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Effect of *Carum carvi* and *Curcuma longa* on hormonal and reproductive parameter of female rats

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Abstract

Background: Rhizome of *Curcuma longa* and seeds of *Carum Carvi* a folk medicinal plant used as have antifertility potentials. Present investigation study the effect of both the plant on hormone and reproductive parameter of female rat. The aqueous and ethanolic extract of rhizome of *Curcuma longa* and seeds of *Carum Carvi* used for testing antifertility activity in female rat. Aqueous and ethanolic extract of plants were administered orally to female rat for 30 consecutive days. Estrous cycle, reproductive hormones (LH, FSH and estrogen) and weight of reproductive organ were studied in both control and extract-administered groups by using standard methods

Results: The female albino rats after oral administration of different doses of aqueous and ethanolic extracts of Carum *carvi* and *Curcuma longa*, showed a significant antifertility activity. FSH and LH level was significantly decreased in both drugs while amount of estrogen in ethanolic extract of both the drugs treated animals was found to be increased.

The blockage of estrus phase induced by treatment of aqueous and ethanolic extract of both drug. They also increase the weight of ovary, uterus and body weights while uterine weight in immature rats increased in extract treated group.

Conclusions: The present study which was undertaken to find endocrinological and physiological changes in the reproductive system of female albino rats after oral administration of different doses of aqueous and ethanolic extracts of Carum *carvi* and *Curcuma longa*, showed a significant antifertility activity.

Keywords: Antifertility; Estrogenic, Reproductive, *Curcuma longa* and *Carum carvi*

Introduction

Ancient Indian literature abounds information on a large number of plants reported to have abortifacient, contraceptive and sterilizing properties and scholars of Ayurveda have also mentioned several plants preparations. Medicinal plants have been used by the

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women of rural communities and especially by tribals to prevent conception.

Curcuma longa Linn. (Zingiberaceae), is commonly known as Haldi, commonly used by all people. It posses Anti-inflammatory [1], Hypolipidemic [2], Anti-cancerous [3] Antifertility activity [4] reported that curcuma extract possess significant anti-fertility and anti-spermatic activity in male albino rats. But there are no study on effect of *Curcuma longa* extracts hormone and reproductive parameter of female rat. *Carum carvi* (CC) (Apiaceae), locally known as 'Kala Jira'. It posses antibacterial [5], antiulcerogenic [6], antitumor [7], antiproliferative [8] and antihyperglycemic [9].

Both plants was used by local tribal people as antifertility agent in females. The present study was undertaken to find out the unexplored antifertility and hormonal activities of the alcoholic and aqueous extract of the rhizome of *Curcuma longa* and seeds of *Carum carvi.*

Materials and methods

Procurement and Rearing of Experimental Animal

Albino rat (Sprague dawley) used in the present investigation was procured from Indian Veterinary Research Institute Jabalpur (MP) .The rats were acclimatized for 15 days to the best laboratory conditions prior to experiments and were maintained on the balanced diet, (Hindustan lever Ltd.). Water was provided *ad libtum*.

Collection, Drying and Storage of Drug

Rhizome of *Curcuma longa* and seed of *Carum carvi* were purchased from the local market and rhizome of *Curcuma longa* arranged between the layers of calcium hydroxide (lime stone) , which were then soaked with water in an earthern pot . After covering the mouth of the pot with cloth, it kept in shade for a month. After a month the mixture of rhizome and calcium hydroxide was separated, rhizome were washed and dried under shade, which acquire dark brown to black colour after reacting with calcium hydroxide.

Dried treated rhizome of *Curcuma longa and Carum carvi* seeds were powered mechanically, weighed and placed in small plastic bags and stored until use.

Extraction of plant material

The powdered seeds of *Carum carvi* and Calcium oxide treated rhizome of *Curcuma longa* were extracted successively with ethanol and distilled water for 20-22 cycles of each after defeating of powdered drug with petroleum ether. The extracts were concentrated to dryness in a vacuum evaporator. Extract were weighed and kept at 4°C in refrigerator until the experimental testing .

Phytochemical studies

Phytochemical studies of the extract were carried out using qualitative and thin-layer chromatography (TLC) methods [10].

Screening of Antifertility Activity

For the evaluation of antifertility activity of different plant extract following studies was carried out. Determination of reproductive phase, effect on estrus cycle, study of biochemical parameters FSH, LH and estrogen level, Estrogenic/antiestrogenic activity of the extract of plants, anti-ovulatory activity [11].

Preparation of Test Drug /Dose

For antifertility studies, concentrated ethanolic and aqueous extracts were freshly prepared and dispersed in distilled water/ethanol containing 0.5% Carboxy methyl cellulose CMC as suspending agent. Ethanolic and aqueous extracts of *Carum carvi* and *Curcuma longa* were prepared at different concentrations and administered orally.

Preparation of Vaginal Smear

For taking vaginal smear, method of Hafez, 1970 [12] was followed. The animals were held with ventral side up. A drop of 0.9% w/v normal saline was inserted carefully in to the vagina with a dropper, without damaging the vagina to avoid false positive smears. The drop of normal saline was aspirated and introduced twice, before withdrawing from vagina the withdrawn fluid was transferred on to a microscopic glass slide .A cover slip was placed carefully on the smear avoiding the entry of air bubbles. The slide was then observed under an optical microscope.

Effect on hormone and female reproductive system

The studies were conducted on adult female rats (140-160g) for 30 days. To study the cycle pattern, animal showing regularity in three normal cycles were separated and chosen for further studies. Those animals showing normal estrus cycles were divided in

to 18 groups of 6 animals each , drugs were ingested orally after mixing in the standard pallet diet . Animals of control group were given standard pellet diet (Hindustan Lever Ltd., India). Synthetic drug, ethinyl estradiol was given as synthetic drug for comparison. Vaginal smear using saline solution were taken twice daily during the entire treatment period (Table 1). Animals were sacrificed 24 hrs. after the last dose .Ovary and uterus were dissected out from adhering tissues, adhering moisture was removed by pressing gently between two layers of filter papers and were immediately weighed on single pan electronic balance(Table 2) . For biochemical studies the blood was collected by cardiac puncture or retro-orbital. The collected blood was used for estimating serum FSH, LH and estrogen level (Table 3).

Estrogenic activity

Immature female rats (22-25 days old) weighing 32-35 g were used in studies. Animals were housed under the uniform husbandry condition as described earlier. They were divided into 18 groups of 6 animals in each group. Drugs were ingested orally and animals of control group received normal diet. Animals were sacrificed 24 hrs .after the last dose. Uterine were dissected out from adhering tissues. Adherent moisture was removed by passing gently between two layers of filter papers and was immediately weighed on single pan (electronic) balance (Table 4).

Statistical analysis

The data were statistically analyzed and expressed as mean ± SE. Statistical analysis of the variance between control and experimental values was done using Student's t test

Results

Phytochemical studies

Various test for analysis of different constituents of plant material such as fixed oils and fatty acids, alkaloids, carbohydrates and glycosides, phytosterols, steroids, tannins phenolic compounds and proteins were performed . Out of those ethanolic extracts of both the plants showed positive test for alkaloids, carbohydrates and fixed oils and fats. While aqueous extract of *Carum carvi* and *Curcuma longa* showed positive test for carbohydrates, proteins and glycosides. Saponins were found to be present in all the extracts except ethanolic extract of *Carum carvi.*

Table 1: Effect of *Curcuma longa* and *Carum carvi* extracts on Estrus Cycle

Treatment	Dose mg/kg for 30 days	Status of Vaginal opening (%) (Approximately)	Status of Vaginal Smear
Control	Normal diet	-	-
CLEE	150	50	PE/E
CLEE	200	75	PE/E
CLEE	250	100	PE/E
CLEE	300	95	PE/E
CLAE	150	0	ME
CLAE	200	0	ME
CLAE	250	73	PE/E
CLAE	300	90	PE/E
CCEE	150	0	ME
CCEE	200	60	PE
CCEE	250	80	PE/E
CCEE	300	95	PE/E
CCAE	150	0	ME
CCAE	200	0	ME
CCAE	250	50	PE/E
CCAE	300	85	PE/E

Curcuma longa ethanolic extract- CLEE, *Curcuma longa* aqueous extract- CLAE, *Carum carvi* ethanolic extract-CCEE, *Carum carvi* aqueous extract-CCAE, E – Estrus, ME –Metaestrus, PE –Proestrus

Effect on hormone and female reproductive system

The present study which was undertaken to find endocrinological and physiological changes in the reproductive system of female albino rats after oral administration of different doses of aqueous and ethanolic extracts of *Carum carvi* and *Curcuma longa*, showed a significant antifertility activity .In all animals slight to significant increase in weight of ovary , uterus and body weight was observed.

Table 2: Effect of *Curcuma longa* and *Carum carvi* extracts on uterus, ovary and body weight

Treatment	Dose mg/kg	Body weight(mg)	Ovary (mg)	Uterus (mg)
Control	Normal diet	150.6 ± 11.6	25.3 ± 0.11	92.8 ± 5.4
CLEE	150	152.9 ± 8.9	41.9 ±7.13	207.4 ± 6.3
CLEE	200	165.1 ±10.2*	62.5 ± 90.10**	248.2 ± 7.1
CLEE	250	173.9 ± 9.1**	76.0 ± 6.12**	287.4 ±10.5
CLEE	300	167.9 ± 10.1**	72.8 ± 8.12**	285.5 ±10.9
CLAE	150	152.0 ± 8.8	31.9 ± 5.14	107.21 ± 9.9
CLAE	200	160.1 ±10.1	37.0 ± 4.15	117.0 ±10.8
CLAE	250	165.2 ± 7.7*	48.6 ± 5.10*	221.9 ± 6.8
CLAE	300	168.8 ± 11.0*	52.9 ± 7.13*	230.11 ±110
CCEE	150	152.3 ± 9.9	31.8 ± 6.11	102.9 ± 9.5
CCEE	200	161.7 ±10.1*	59.0 ± 4.12*	210.2 ± 5.6
CCEE	250	165.1 ± 11.7*	62.8 ± 7.11**	255.1 ± 9.8
CCEE	300	172.1 ± 9.1 **	70.0 ± 8.91**	271.8 ± 8.8
CCAE	150	151.9 ± 8.3	29.8 ± 9.12	112.9 ± 8.9
CCAE	200	159.7 ± 7.9	31.19 ± 5.13	148.6 ± 7.1
CCAE	250	161.2 ± 9.3*	42.8 ± 5.15*	169.1± 2.9
CCAE	300	169.2 ±8.6**	49.9 ± 4.14	185.5 ± 7.4
Ethinyl estradiol	0.1 µg/ IM	179.9 ±8.9**	83.2 ± 6.81**	291.2 ± 9.2

Mean ± S.E. ; * P< 0.05. ; ** P<0.001

Table 3: Effect of *Curcuma longa* and *Carum carvi* extracts on hormone levels

Treatment	Dose mg/kg	FSH (Pg/ ml)	LH (Pg/ ml)	Estrogen (Pg/ ml)
Control	Normal diet	0.188 ± 0.003	0.15 ± 0.015	42.23 ±1.62
CLEE	150	0.169 ± 0.005	0.14 ±0.009	56.30 ± 1.69
CLEE	200	0.139 ±0.006	0.10 ± 0.08	62.11 ± 1.81
CLEE	250	0.120 ± 0.009*	0.07 ± 0.01**	69.21 ± 2.99**
CLEE	300	0.125 ± 0.007*	0.08 ± 0.05*	61.33 ±1.79**
CLAE	150	0.180 ± 0.004	0.14 ± 0.051	43.99 ± 2.51
CLAE	200	0.161 ± .0112	0.10 ± 0.31	54.29 ± 1.99
CLAE	250	0.151 ± .010	0.10 ±0.04	59.32 ± 2.00*
CLAE	300	0.132 ± 0.009*	0.08 ± 0.011*	68.91 ± 2.09**
CCEE	150	0.188 ± 0.099	0.14 ± 0.018	46.59 ± 1.91
CCEE	200	0.149 ± 0.12	0.12 ±0.19	57.37 ± 2.01*
CCEE	250	0.130 ± 0.001*	0.09 ± 0.021*	61.31 ± 1.99**
CCEE	300	0.125 ± 0.09*	0.08 ± 0.023*	68.18 ± 2.10**
CCAE	150	0.187 ± 0.005	0.14 ± 0.076	43.01 ± 1.99
CCAE	200	0.181 ± 0.019	0.14 ±0.99	49.31 ± 2.01
CCAE	250	0.177 ± 0.05	0.12 ± 0.0099	55.21 ± 1.99*
CCAE	300	0.139 ±0.02*	0.09 ±0.0031*	59.20 ± 1.15*
Ethinyl estradiol	0.1 µg/ IM	0.119 ± 0.008*	0.02 ± 0.11*	77.71 ± 1.9**

Mean ± S.E. ; * P< 0.05. ; ** P<0.001

Table 4: Effect of *Curcuma longa* and *Carum carvi* extracts

Treatment	Dose mg/kg for 30 days	Uterine weight mg/100g
Control	Normal diet	73.9 ± 4.4
CLEE	150	102.4 ± 7.3
CLEE	200	230.2 ± 7.1*
CLEE	250	268.5 ± 10.6*
CLEE	300	264.5 ± 9.9**
CLAE	150	83.3 ± 8.9
CLAE	200	92.5 ± 9.1
CLAE	250	165.7 ± 5.9*
CLAE	300	191.3 ± 8.1
CCEE	150	82.9 ± 8.5
CCEE	200	181.7 ± 6.6*
CCEE	250	231.2 ± 8.8*
CCEE	300	250.8 ± 7.8**
CCAE	150	80.7 ± 6.9
CCAE	200	189.5 ± 8.1
CCAE	250	140.2 ±5.9
CCAE	300	183.4 ± 5.4*
Ethinyl estradiol	0.1 µg/ IM	270.2 ± 8.3**

Mean ± S.E.; * P< 0.05. ; ** P<0.001

Ethanolic extract at various dose level of *Curcuma longa* administered orally for 30 days, exhibited its effect on ovarian endocrinology i.e. FSH, LH quantities. Both the gonadotropins FSH and LH were measured after 24 hours of the last dose of the drug. The unit of both the hormones in per ml. volume of serum showed a declining change .This change gradually increased with the increased dose of the drugs. The lowest concentration with any of the extract, whether aqueous or ethanolic, recorded with ethanolic extract of *Curcuma longa* at 250 mg doses, of FSH was 120pg / ml blood and LH was 0.7 unit in the same volume of blood. Aqueous extract of the same herb although followed the same manner as that of its ethanol extract yet the effect is not so much prominent .The interesting fact is observed with ethanolic extract is that at 300 mg a negative effect is started as compared to the dose at 250 mg ethanolic extract of the same drug. The ethanolic and aqueous extract of *Carum carvi* at initial doses does not seem to have any effect on FSH and LH. With increasing dose of ethanolic extract, the amount of both gonadotropins, FSH and LH, declines but their concentration was not so much significant with aqueous extract treated animals. The amount of estrogen in ethanolic extract of both the drugs treated animals was found to be increased. Comparatively, it was quite high in *Curcuma longa* than that of *Carum carvi*.

Another effect is observed in with the weight of uterus, which is recorded the highest in *Curcuma longa* treated animal among any other extract treated animals. For the study of estrus cycle, oral administrations of ethanolic and aqueous extracts were done. Results obtained by the study indicate blockage of the sequential changes in the vaginal smear in the estrus or proestrous phase induced by treatment of aqueous and ethanolic extract of *Carum carvi* and *Curcuma longa* .The table 1 showing the status of reproductive cycle with extracts (aqueous and ethanolic) of both the plants are actually representing the mean percent of vaginal opening. The uterus and ovarian weights of adult female rats treated during 30 days of treatment period by various doses of both the plant extracts are given in table 4 while uterine weights of immature female rats after oral administration of the same drugs are given in table 3 for conforming estrogenic effect of the drugs on immature female rat respectively. Significant difference in the weight of ovaries and uteri of treated and control rats were absorbed. *Curcuma longa* showed highest increase in weight of ovary, uterus and body weights at 250mg/kg of ethanol extract, while uterine weight in immature rats increased as 258.5 mg /100g, with the similar dose of the same drug, which was highest in all treated immature rats.

Discussion

All parameters like vaginal opening, smear count and uterine weight were suggesting estrogenic activity of both plant extracts. Saidudin and Zassenhuaus, 1978, in a study on oestradiol receptor in the immature rat ovary, also found that three daily injections of 2.5µg oestradiol significantly increased ovarian as well as uterine weight. Finally it was confirmed by biochemical parameters also. Serum level of estrogen was higher than those of controls. On the contrary, FSH and LH levels of serum were significantly decreased. The drug contains very little amount of a particular compound, which attains a proper level of stimulatory activity, once the drug has been given in appropriate concentration. The compound (s) either may be having direct estrogenic effect or after getting metabolized the products formed might be acting in many ways i.e. the said compound is either estrogen as its affects are confirmed in various ways or activating gonadotrophic inhibitory hormone to prevent the release of corresponding hormones or blocking the receptors present in ovary to prevent the follicle maturation. The compound is mimicking estrogen like shape and stimulating/causing the growth of uterus. Such compounds are presents in plants in appreciable amount. The compound can be converted to estrogen if a proper enzyme system to act upon them is present, whatever it may be, whatever estrogen like compound or its final product after metabolism in body is estradiol. It is beyond any doubt that uterine growth involving the changes in the endometrium, enlargement of blood vessel, increase in amount of connective tissue; all are activated by estradiol. The ethanol extract, which showed stronger activity than aqueous extract, led to assumption that it has cyclopentano-phenethrene like compound or a compound having an impirine chain, which finally produces estrogen like compound. Such types of compound have more solubility in organic solvent than aqueous. Difference in the weight of uterus and amount of FSH and LH assessed strongly point to the fact that the compound present in *Curcuma longa* after treatment with calcium oxide is changed to some steroid and having almost similar configuration as that of ethinyl estradiol. When the rhizome of *Curcuma longa* treated with calcium oxide, good amount of heat is generated in earthen pot, which remains persistent for quite sometime; this causes continuous reaction with calcium hydroxide to generate few compounds, which are not detected in treated dry rhizomes of *Curcuma longa*. Further studies are needed to isolate the components, its nature and its quantities in the extract, which are causing uterotropic activity.

Estrogens are steroid hormones which, together with other hormones, control the ovulatory cycle in the female mammal [13]. Estrogen acts in a feedback mechanism, influencing the production of follicle stimulating hormones (FSH) from the pituitary gland. It is known that the FSH in turn promotes the development of the immature ovarian follicles, which increases the production of estrogen from the ovary. This is readily done if excess exogenous estrogen is administered, thus prevent ovulation by inhibiting the release of the gonadotropin releasing factor from hypothalamus, that is exogenous hormones exert a negative feed back on the hypothalamus in a manner similar to that by the naturally occurring hormones.

As a result of hypothalamic suppression by an oral contraceptive, gonado- tropic output from the pituitary is decreased. In short, if FSH is sufficiently suppressed by the estrogenic compound of the drugs, follicular growth will be minimum. Therefore the occurrence of ovulation would be unlikely even in the presence of the higher leutenizing hormone levels.

Experimental work has suggested that immediately before the surge occurs there is probably a sudden depression of estrogen secretion by the ovarian follicles and that this might be the necessary signal for causing the subsequent positive feedback effect that leads to the surge. Obviously, administration of the sex hormones could prevent the initial hormonal depression that might be the initiating signal for ovulation.

Present findings indicate that the administration of the extracts of both the drugs showed significant increase in the estrogen level and induce inhibitory effect on FSH and LH resulting in failure to ovulate. This can result from hyposecretion of gonadotropic hormones, in which case the intensity of the hormonal stimuli simply is not sufficient to cause ovulation, or it can result from abnormal ovaries that will not allow ovulation.

As indicated by the results of preliminary phytochemical screening of extracts, aqueous and ethanolic extracts of both the plants showed positive

results for glycosides while test for alkaloids were found to be positive in ethanolic extracts, and it is known from previous studies of different authors [14, 15] that alkaloids and glycosides have contraceptive properties. Alkaloids and glycosides mainly flavonoids (16], from plant origin were tested for antifertility efficacy and found to be effective for including estrogenic response and thus having contraceptive activity. In this study, the extracts of the plants exhibited marked uterotrophic activity in treated animals.

Various parts of the different plants contain estrogens and they have their effects on the different life processes. Phytoestrogens are noble estrogens found in variety of plants, which may be ingested directly or as constituents of tissue from animals that have ingested plants. Phytoestrogens have noxious effect leading to impaired fertility in domestic animals as well as disturbance of the normal gestation process [17]. They may affect and regulate the reproduction and reproductive cycle. The most important manifestation of estrogenic activity in plants is due to the presence of isoflavon. *Carum carvi* does have estradiol mimic compounds but either their activity on the target organs like ovary, uterus and pituitary may not be so efficient or their less concentration is responsible for this. Although a flavonoide from *Carum carvi* seeds is already reported for antibacterial activity, these flavonoids may be responsible for estrogenic activity in present study. Preliminary phytochemical screening also revealed the presence of glycosides in both plants of both aqueous and ethanolic extract of the plants. Yet they may possess molecular structure similar enough to estrogen to bind to estradiol receptors. The practical importance of the phytoestrogens lies with their ability to alter the biological response to endogenous estrogens. Estradiol receptors will bind to a diverse group of chemical compounds including other steroids, isolflavones and phytoestrogens. When phytoestrogens bind to estrogen receptors or cells, they translocate to the nucleus and stimulate cell growth in a manner similar to estradiol. Despite the apparently weak relative binding capacity of the phytoestrogens they can have significant hormonal effects, [18]. This is due to their lower affinity for the serum estrogen binding proteins, and resulting in a net effect of enhancing the concentration of available phytoestrogen at the target tissue sites. Plants estrogens bind directly to estrogen receptors and provide estrogenic effect. This is enhanced by the tendency of the phytoestrogens to concentrate. This has been clearly demonstrated in the laboratory that uterine weight assays show effects equivalent to estradiol when sufficient phytoestrogen was used [19]. Alkaloid of plant origin also possesses a frank estrogenic activity, as reported earlier [20] which it may be recalled is found to be present in ethanolic extracts of both the plants in present study.

A typical estrogenic compound possesses ability to increase the uterine weight [21], but a frank estrogenic compound is that which induces cornification and opening of vagina in immature rats. Present study revealed the same findings, therefore it can be concluded from the results of the study that alkaloids present in ethanolic extract of *Carum carvi* and *Curcuma longa* are responsible to induce frank estrogenic activity, as proved by cornification and opening of vagina in immature treated rats. The plant products used in this study contains several compounds, which were previously identified in preliminary qualitative chemical analysis and other compounds with pharmacological importance. Therefore the contraceptive effects observed in this study could be due to one or more of the active constituents of the extracts.

Both the plants extracted have alkaloids and glycosides though these components are not isolated but it can be presumed that these may individually or synergistically affect and uterus.

It is possible that both alkaloids and glycosides may be responsible for infertility in present investigation but alkaloids seem to be stronger to induce contraceptive effect as evident by more pronounced estrogenic activity of ethanolic extracts of both the plants as compared to aqueous extracts and as revised earlier, ethanolic extracts showed positive test for alkaloids in both the plants. In summary, the estrogen-dominated phase cause thickening of the vaginal mucosa and proliferation of the uterine endometrium, this is proved by the results of the present study and indicating the estrogenic nature of the drugs.

Data obtained from the parameters analyzed corroborate with this preliminary conclusion, the serum oestradiol level was high in all treated groups, irrespective of the dose. The drugs as concluded from the results elevated estrogenic activity, which (by feed back) inhibits FSH secretion from pituitary and

therefore prevent the development of new follicle in ovary resulting inhibition of ovulation and impairment of fertility and thus contraception. Therefore, there remains definite scope for further research with these plants as they provide safe contraception, without disturbing general physical conditions, as they did not show any toxic effect in present study.

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