EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 12
Year: 2024

# ANTIFERTILITY ACTIVITY OF AQUEOUS-ETHANOLIC RHIZOME EXTRACT OF *CURCUMA LONGA* IN RATS

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**Article Received on 07/10/2024** **Article Revised on 28/10/2024** **Article Accepted on 18/11/2024**

**ABSTRACT**

The practice of traditional medicine for the control of fertility in most parts of India is based on the uses of plant medicines for many years. The fact that herbal medicines have been employed for such a long time does not guarantee their efficacy and safety. The aim of present study is to evaluate the anti-fertility activity of aquous-ethanolic extract of rhizome of *curcuma longa* in rats. *Curcuma longa* belongs to Family Zingiberaceae.[1] Curcumin is the active ingredient in the traditional herbal remedy and dietary spice turmeric (*Curcuma longa*). The activity was evaluated by studying the effects on estrous cycle, anti-implantation activity, Abortificient activity, and studies on male hormonal changes on continuous treatment of drug.

**KEYWORDS:** Antifertility activity, Curcuma longa, Zingiberaceae, Rhizome, Curcumin.

## INTRODUCTION

NATURE always stands as a goldenmark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all independent. The plants are sindispensable to man for life.[1] The three important necesseties of life- food, clothing, and shelter –and a host of other useful products are supplied to him by the plant kingdom. Nature has provided a complete store-house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a results of man's inquisitive nature so that today we possess many effective means of ensuring health- care. Natural plant products have been used throughout human history for various purposes; hundreds of studies were conducted to investigate the effects of natural origin compounds on human health and prevention and treatment of dieses.[2] India is known as the "Emporium of medicinal plants". The country also has to its credit that well known traditional systems of medicine like Ayurveda and Siddha. These systems of medicine derive their drugs primarily from plant origin. The World Health Organization (WHO) has also recognized the traditional systems of medicine as one of the tools to achieve its aim "Health for all". Many indigenous plants posses the property of preventing conception orally. Such plants have been tested for their anti-fertility activity in laboratory animals. Various extracts were used in the investigation to determine anti-fertility activity using a method which would ultimately detect anti-zygotic, blastocystoxic, anti-implantation or early abortifacient activity in female rats of albino strain of proven fertility following successful matting. Birth control methods have been used since ancient times but effective and safe methods only became available in the 20th century. Some cultures deliberately limit access to contraception because they consider it to be morally or politically undesirable. One of such important species is Rhizome of *Curcuma longa*, a folk medicinal plant has found to possess anti-fertility potentials. Focusing on Indian traditions, we find a preferable use of turmeric to abort fetus. Based on this traditional claim, present investigation study the effect of both the plant on hormone and reproductive parameter of female rat.[2] The aqueous-ethanolic extract of rhizome of *Curcuma longa* used for testing anti-fertility activity in female rat. Aqueous-ethanolic extracts in various concentrations of plant 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 Curcumin is a free radical scavenger and hydrogen donor, and exhibits both pro- and antioxidant activity. Hypothesis suggests Curcumin induces DNA

fragmentation and base damage in the presence of copper and isozymes of cytochrome p450 (CYP) that may be responsible for Chromosomal abnormalities, sperm DNA damage, zona hardening, inadequate culture conditions, and suboptimal embryo development all play a significant role in the etiology of recurrent implantation failure. Evidence suggests that pre-implantation genetic screening does not increase implantation or live birth rates. This hypothesis focuses on a great research work towards the unrevealed activities which can be shown by curcumin and a promising future in medicinal research. Development of new contraceptives for the female depends on a thorough knowledge of the anatomy and physiology of the reproductive tract. The female has five major roles in reproduction: (1) developing a gamete (ovum); (2) receiving the male gamete (spermatozoa) and transplanting them from the vagina to the distal portion of the oviducts; (3) supplying a suitable milieu for fertilization; (4) directing the fertilized ovum across the fallopian tubes to the uterine cavity; and (5) providing a uterine environment conductive to implantation and maintenance of gestation. These functions provide an excellent opportunity for biologic and pharmacologic manipulation of the female reproductive process to achieve fertility control. On the other hand, it has been reported that 50% of ethanolic extracts of *curcuma longa* at the dose of The whole herb of curcuma longa contains most of the phyto chemical constituents which may responsible for the anti-fertility activity in both male and female such as glycosides, flavonoids, etc. thus, the present study deals with the screening of anti-fertility efficacy by using different experimental models. Preparations of water-soluble curcumin by incorporation into various surfactant micellar systems (acetone, methanol, and ethanol) have been reported.[3] It is stable at high temperatures and in acids, but unstable in alkaline conditions and in the presence of light. The antioxidant property of curcumin can prevent rancidity of foods and provide foodstuffs containing less oxidized fat or free radicals. The powerful anti-oxidation property of curcumin has an important role in keeping curry for a long time without it turning rancid. Curcuminoids are poorly soluble in the hydrocarbon solvents. Curcumin is an oil soluble pigment, practically insoluble in water at acidic and neutral pH, soluble in alkali. Curcumin is widely used to colour many foods.[4] Curcumin is listed for use in dairy products, fats, oils and fat emulsions, edible ices, fruit and vegetable products, confectionery, cereal products, bakery wares, meat and meat products, fish and fish products, eggs and eggs products, spices, soups, sauces and protein products,

## METHODOLOGY

### Procurement and rearing of experimental animal

A specific pathogen free healthy wistar albino rats were obtained (Regional Cancer Research Institute of Gwalior, Madhya Pradesh) and acclimatized to the laboratory conditions (in ADINA Institute of Pharmaceutical Sciences, Sagar, Madhya Pradesh). They were housed under conventional conditionin polypropylene cages in a controlled environment. They were provided with water and food before starting experiment. Experimental protocol was approved by the institutional ethical committee.

### Collection, drying and storage of drug

Rhizome of *Curcuma longa* 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 earthen 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* were powered mechanically, weighed and placed in small plastic bags and stored until use.

### Extraction of plant material

The powdered 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 evaluation was performed. Both qualitative and quantitative phyto-chemical evaluation was performed for detection of various phyto-constituents like alkaloids, glycosides, amino-acids, carbohydrates, etc. some of them were found to be present. Out of that, the two main phyto-constituents which were found to present and had a great significance were phenolics and flavonoids. Further quantitative evaluation for both the constituents was performed.

### Thin layer chromatography

For thin layer chromatography, pre-coated silica gel F-254 aluminium plates (20*20 cm) were used. The curcumin from *curcuma longa* was separated using n-hexane.

## EXPERIMENTAL STUDIES

### Acute toxicity studies

For the purpose of acute toxicity studies of Hydro-ethanolic *curcuma longa* rhizome extract (HECLR), They were housed under conventional conditionin polypropylene cages in a controlled environment. They were provided with water and food before starting experiment. Experimental protocol was approved by the institutional ethical committee. Selected animals were divided in four groups and each consisted of 5 wistar rats. The animals of I,II and III group were fed with the extract (HECLR) orally in increasing dose levels of 500, 700, and 1000mg/kg body weight and controll group received normal saline 2 ml/kg. The animals were

observed for their behavioural response, neurological responses and autonomic profile. The intensive observations were taken for next 7 days, and further anesthetized for blood collection for hematological parameters.

**Preparation of testing dose**

Concentrated hydro-ethanolic dose was prepared freshly and dispersed in ethanol containing 0.5% carboxy methyl cellulose CMC as suspending agent. And then extracts were prepared in different concentrations for oral administration of animals.

**Pharmacological screening of reproductive parameters**

**1. On Female reproductive parameters**

**1.1 Estrogenic activity**

Immature female rats (22-25 days old) weighing 32-35 g were taken.animals were housed under uniform husbandry condition. they were divided into 4 groups of 3 animals each. Further drug was administered orally to the animals of 3 groups and 4$^{th}$ group was considered to be control group and treated with saline. After 24 hours, animals were anasthetized and uterine was dissected out from adhereing tissues and moisture was removed by passing gently between two layers of filter paper and immediately weighed on single electronic balance.

***Effect on hormones and reproductive parameters***

For the evaluation of anti-fertility activity, determination of reproductive phases, effect on estrous cycle, study of biochemical parameters like alkaline phosphatase enzyme determination, glycogen concentration determination and cholesterol determination, FSH, LH and estrogen level is studied.

**1.2 Anti-fertility activity**

The animals were exposed to altermate cycle of 12 hrs. day and night each. before each test animals were fasted for 12 hrs. Prior to test. The experimental protocols were subjected to securitization of the institutional animal ethics committee and were cleared by the same (**1546/PO/a/11/CPCSEA**). Except for the experiment on estrogenic activity, sexually matured rats were taken and provided with commercial standard food and water *ad.libitum*.

**Grouping of animals-** 15 sexually matured female rats were taken and grouped in following pattern.

| S. No | Group number | Name of group |
|---|---|---|
| 1 | Group 1 | Anti-conceptive group |
| 2 | Group 2 | Abortifacient group |
| 3 | Group 3 | Served as control |

**Procedure**

In the experiment to determine the above activities, before grouping animals, each female rat was paired with male animal and allowed to stay for the overnight. Next morning mating was confirmed with the help of observation of plug on the floor and also by observation of slides in the microscope. As the mating was confirmed, females were separated and allowed to stay in separate cages and the day was considered as the day 1 of pregnancy after mating.

**a. Anti-conceptive activity**

From above pregnant rats, a group of 5 animals received 250 mg/kg of HECLR extract from day 1 to day 4. This was regarded as anti-conceptive group.

**b. Abortifacient activity**

Another group of 5 rats was allowed to administer 250 mg/kg of the drug extract from day 8 to day 11. This group was regarded as abortifacient group.

**1.3 Anti-Implantation Activity**

All the animal**s** were maintained under controlled standard animal house condition and provided with food and water *ad libitum*. Female rats were observed for oestrous cycle and phase was determined through smear count. Female rats who were in their pro-estrous phase were kept with male rats of proven fertility for mating. After confirmation of mating, pregnant female rats on day 1 were divided into 3 groups of 5 rats each.

**Drug administration and observation**

Extracts in different concentration were administrated orally from day 1 to day 7 of gestation. The control animals received only vehicle. On the 10$^{th}$ day, laparotomy was carried out under light ether anaesthesia in semi-sterile condition. The uteri were examined to determine the number of implantation sites. The number of corpora lutea in ovaries was also recorded. The abdomen was sutured animals were left in cages. The drugs were administered to the animals orally again for 3 days (day 14 to 16). On the 18$^{th}$ day, laparotomy was carried out once again under light ether anaesthesia for abortifacient activity.

The percentage of anti-implantation and early abortifacient activity was calculated by using following formulae.

$$\psi\ of\ anti-implantation\ activity = 100 - \frac{no.of\ implantations}{no.of\ corpora\ lutea} \times 100$$

$$\psi\ of\ abortifacient\ activity = \frac{no.of\ resorptions}{no.of\ corpora\ lutea} \times 100$$

**2. On Male reproductive parameters**

**2.1 Aphrodisiac activity**

***Animal grouping and extract administration***

A total of 20 male rats were used and were completely randomized into 4 groups A,B,C and D of 5 animals each after being allowed to acclimatized for two weeks. The distilled water and the extracts were orally administered as follows:

Group A: Control group

Group B: 1 ml of the extract corresponding to 200mg/kg body weight

Group C: 1 ml of the extract corresponding to 250 mg/kg

body weight
Group D: 1 ml of the extract corresponding to 300 mg/kg body weight

After administration of above extract and distilled water on daily basis at morning time. The animals were given free access to food and water upto 21 days. In between 1st, 7th and 21st dose 5 rats from each group were sacrificed. The study was approved by the ethical department committee on the care and use of experimental animals. Further quantitative determination of hormones from laboratory i.e. serum progesterone, FSH and LH level was determined.

### 2.2 Androgenic Parameters

15 animals were divided into 3 groups of 5 animals each and following experiments were performed.

The testis-body weight ratio was determined according to (). The concentrations of protein, total cholesterol, Glycogen and sialic acid in the testis were estimated by the methods described by (). Testerone concentration were also determined according to.

## RESULT AND DISCUSSION

Various test for analysis of different constituents of plant material was performed. Out of those ethanolic extracts of plant material following metabolites were found to be present in the extract. *Curcuma longa* showed positive results for:

1. Carbohydrates
2. Saponins
3. Proteins
4. Glycosides
5. Phenolics
6. Flavonoids

**Table 1: Quantitative determination of Phenolics and Flavonoids.**

| Extract | Flavonoid mg/g | Phenolic mg/g |
|---|---|---|
| Hydro-ethanolic extract of *curcuma longa* | 26.12±2.54 | 53±3.21 |

**Studies on female reproductive parameters**

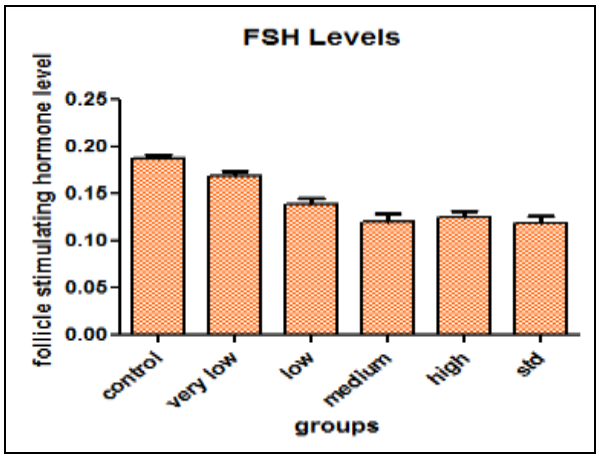
**1. Estrogenic activity**

**Table 2: Effect of curcuma longa extract on hormonal level of female reproductive system.**

| Treatment | Doses mg/kg | Follicle stimulating hormone Pg/ml | Leutinizing hormone Pg/ml | Estrogen Pg/ml |
|---|---|---|---|---|
| Control | Normal diet | 0.188±0.003 | 0.15±0.015 | 42.23±1.62 |
| HECLR | 150 | 0.169±0.005 | 0.14±0.009 | 56.30±1.69 |
| HECLR | 200 | 0.139±0.006 | 0.10±0.008 | 62.11±1.81 |
| HECLR | 250 | 0.120±0.009* | 0.07±0.01* | 69.21±2.99* |
| HECLR | 300 | 0.125±0.007* | 0.08±0.05* | 61.33±1.79* |
| Ethinyl estradiol | 0.1 µg/IM | 0.119±0.008* | 0.02±0.11* | 77.71±1.9* |

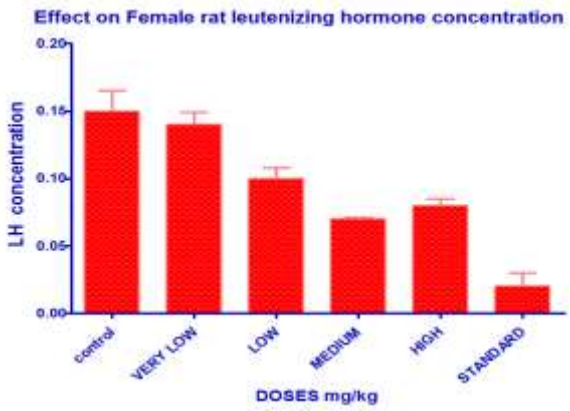
MEAN ± S.E.; *P<0.05; **P<0.001

Animals were given treatment for 30 days. As per the results obtained – as compared to control group, Standard treated (ethinyl estradiol) was found to contain a less amount of FSH then any other group and extract treated (with different doses) showed a sequential decrease in hormonal levels. A little increment was found at high dose (i.e 300mg/kg)(graph 1).



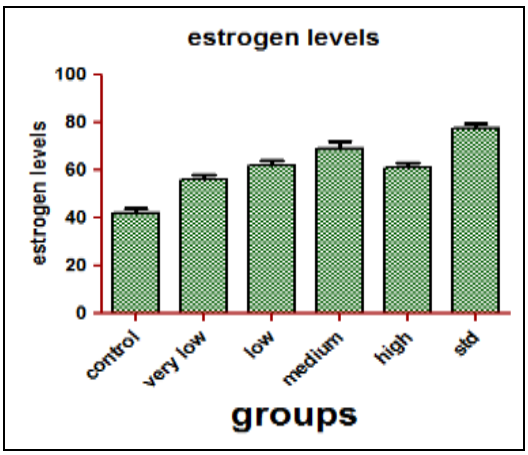

**Graph 1: Graph showing effect of HECLR on Follicle Stimulating Hormone when treated in different doses.**

Similarly pattern for LH, as FSH was observed. As per the results obtained – as compared to control group, Standard treated (ethinyl estradiol) was found to contain a less amount of LH then any other group and extract treated (with different doses) showed a sequential decrease in hormonal levels. A little increment was found at high dose (i.e 300mg/kg) (graph 2)




**Graph 2: graph showing effect of HECLR extract on Luteinizing Hormone when experimental animals were treated in different doses.**

Estrogen levels were found to be very less in control group animals they and maximum in standard group animals. However, as the dose was increased in animals estrogen levels were found to be dependently increasing till moderate doses. Higher dose treated animals showed a decrease in the estrogen levels. (Graph 3)



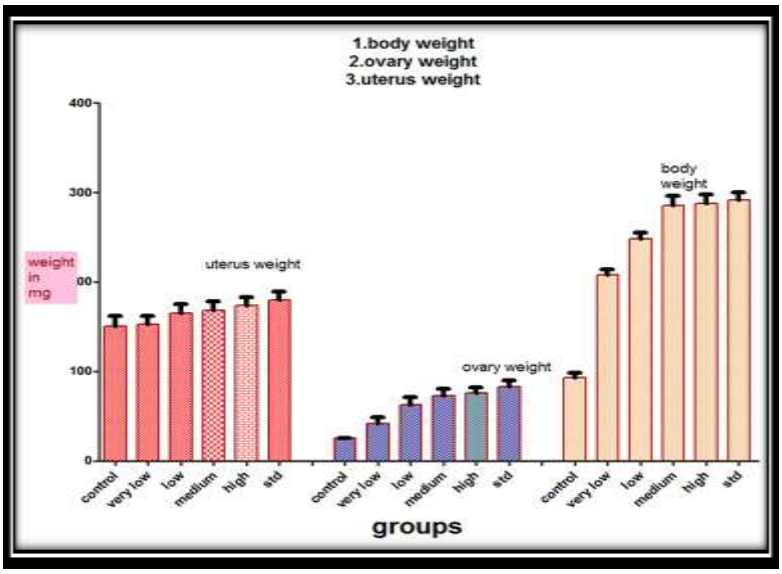

**Graph 3: graph showing effect of HECLR extract on Estrogen level when treated in different doses.**

The results show that there is a significant decrease in the levels of FSH and LH levels with increasing concentration of the drug in female wistar rats. At the same time, an increase in the estrogen level was detected. This gives a direction to the effectiveness of the drug towards the activity.

**Table 3: Effect of *curcuma longa* hydro-ethanolic extract on uterus, ovary and body weight of experimental animals.**

| 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 |
| HECLR | 150 | 152.9±8.9 | 41.9±7.13 | 207.4±6.3 |
| HECLR | 200 | 165.1±10.2* | 62.5±9.10** | 248.2±7.1 |
| HECLR | 250 | 167.9±10.1** | 72.8±8.12** | 285.5±10.9 |
| HECLR | 300 | 173.9±9.1** | 76.0±6.12** | 287.4±10.5 |
| Ethinyl estradiol | 0.1 $\mu g/IM$ | 179.9±8.9** | 83.2±6.81** | 291.2±9.2 |

**Mean ± S.E.;*P<0.05; **P<0.001**




**Graph 4: graph showing effect of HECLR extract on Body Weight, Uterus Weight and Ovary Weight on treating experimental animals with different doses.**

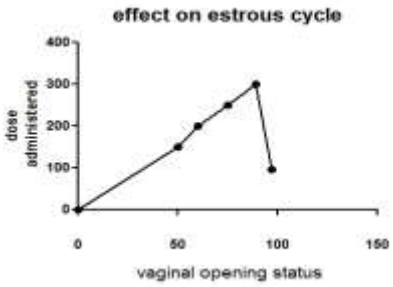
And so are the body weight, ovary and uterus weight which were found to be dose dependently increasing, although there was a very less difference between responses of medium and high dose of extract.

**Table 4: Effect of *curcuma longa* hydro-ethanolic extract on estrous cycle of experimental animals (for 30 days).**

| Treatment | Dose mg/kg | Status of vaginal opening (%) approximately | Status of vaginal smear |
|---|---|---|---|
| Control | Normal diet | - | - |
| HECLR | 150 | 50 | PE/E |
| HECLR | 200 | 60 | PE/E |
| HECLR | 250 | 75 | PE/E |
| HECLR | 300 | 89 | PE/E |
| Ethinyl estradiol | 0.1 $\mu g/IM$ | 97 | |

**E-Estrous phase; PE-Pro estrous phase**

When the doses exceeded of aqueous-ethanolic extract, from very low to high, a change in vaginal opening status was noticed. A percentage increase was found which was maximum at even higher doses. It was found to be 89% at the dose of 300 mg/kg of dose. Which was quite closer to standard drug treated animals which were 97%. This means when increased dose was given to the experimental animals, increasing amount found to showed directly proportional upto limit of 250 mg/kg and reverse results started to reveal as the dose was increased.




**Graph 5: graph showing effect of HECLR extract on estrous cycle on treating experimental animals with different doses.**

**Table 5: Effect of *curcuma longa* hydro-ethanolic extract on anti-implantation and early abortifacient activity.**

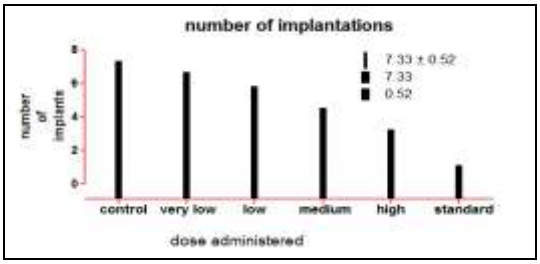
**I. Anti-implantation activity**

| Treatment | Dose mg/kg body weight | Number of implants | Number of litters | Mean % anti-implantation |
|---|---|---|---|---|
| Control | Normal diet | 7.33±0.52 | 7.50±0.55 | Nil |
| HECLR | 150 | 6.67±0.41 | 6.12±0.55 | 10.07±0.39 |
| HECLR | 200 | 5.81±0.39 | 5.69±0.21 | 26.21±0.14 |
| HECLR | 250 | 4.52±0.43 | 4.23±0.19 | 61.21±0.23 |
| HECLR | 300 | 3.25±0.20 | 3.11±0.22 | 34.17±0.34 |
| Ethinyl estradiol | 0.1 $\mu g/IM$ | 1.13±0.12 | 1.01±0.06 | 89.78±0.21 |

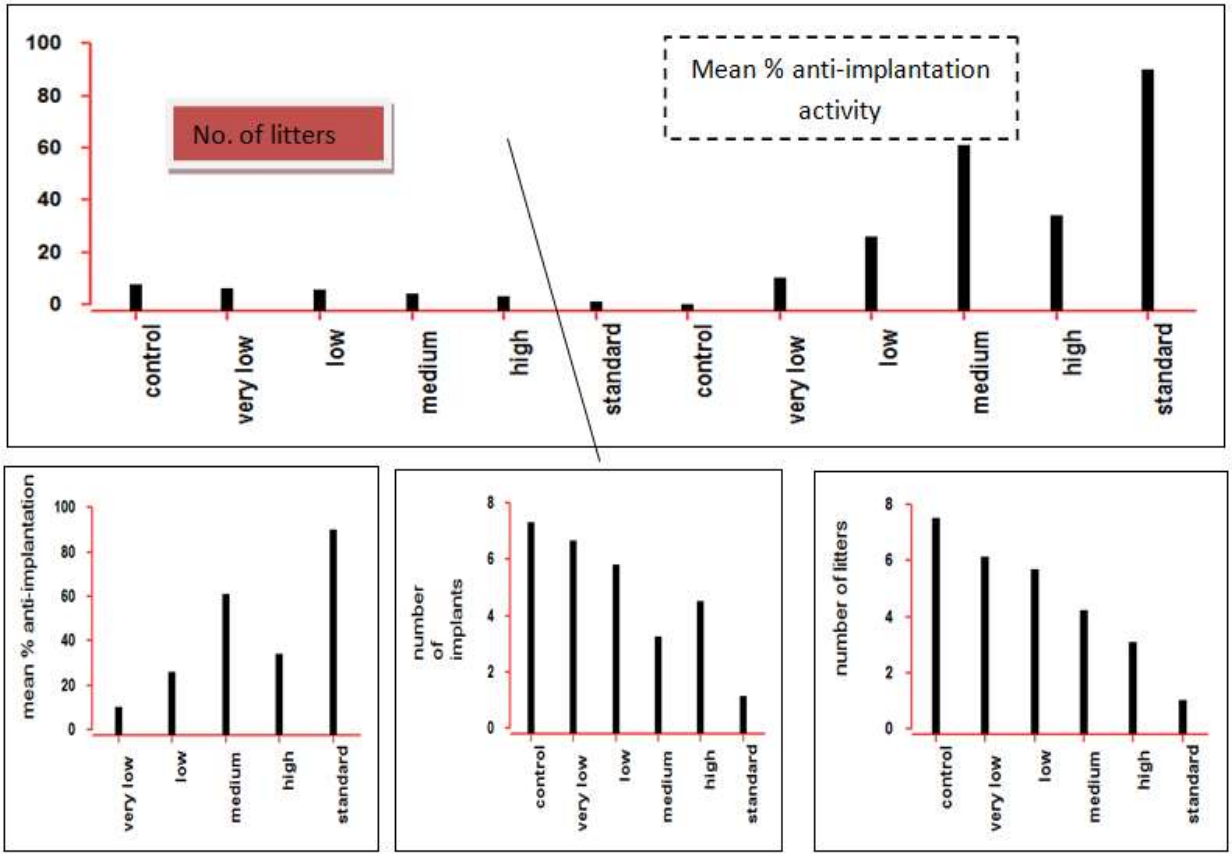
Values are expressed as mean ±S.E.M.P values a=P<0.05

**II. Early abortifacient activity**

| Treatment | Dose mg/kg body weight | Number of implants | Number of litters | Number of resorbed implants |
|---|---|---|---|---|
| Control | Normal diet | 7.33±0.52 | 7.50±0.55 | - |
| HECLR | 150 | 6.67±0.41 | 6.12±0.55 | - |
| HECLR | 200 | 5.81±0.39 | 5.69±0.21 | 1 |
| HECLR | 250 | 4.52±0.43 | 4.23±0.19 | 2 |
| HECLR | 300 | 3.25±0.20 | 3.11±0.22 | 1 |
| Ethinyl estradiol | 0.1 $\mu g/IM$ | 1.13±0.12 | 1.01±0.06 | 3 |




**Graph 6: Graphs a and b showing effect of HECLR extract on anti-implantation activity on treating experimental animals with different doses.**




**Graph 7: Graphs a, b and c showing effect of HECLR extract on early abortifacient activity on treating experimental animals with different doses.**

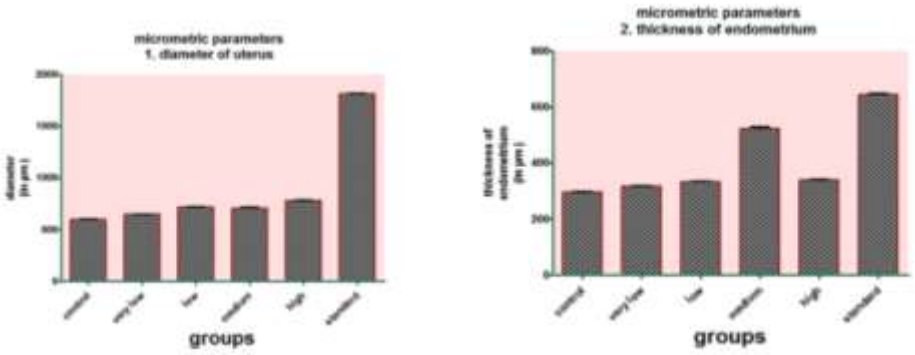
On performing experiment on anti-implantation activity, it was observed that the number of littersincreased with increase in the dose of the drug and further when drug increased from 250 mg/kg, activity was decreased. Now, further based on the number of implantations and number of litters, calculation of mean % anti-implantation was obtained in the same pattern.

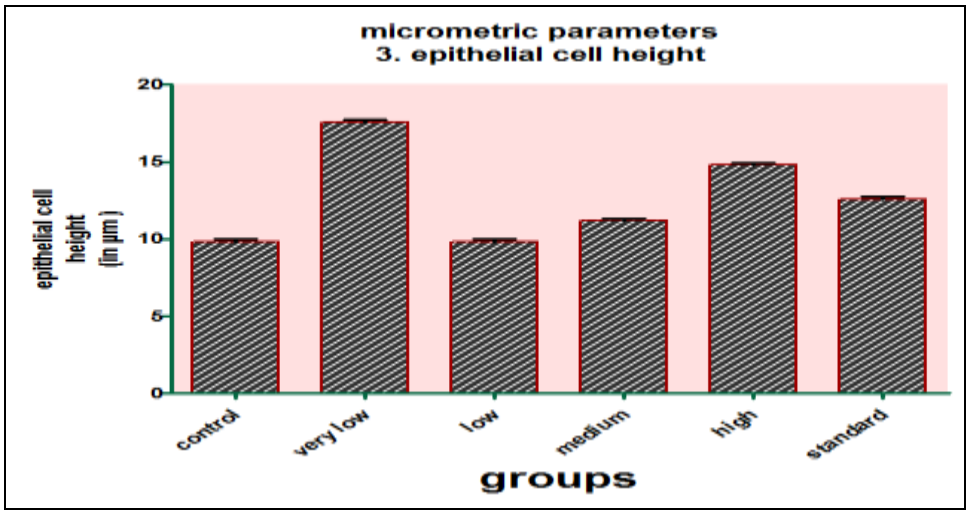
**Table 6: Effect of *curcuma longa* hydro-ethanolic extract on micrometric changes in the uterus.**

| S. No | Treatment | Dose (mg/kg) | Diameter of uterus ($\mu m$) | Thickness of endometrium ($\mu m$) | Epithelial cell height ($\mu m$) |
|---|---|---|---|---|---|
| 1 | Control | TWEEN 80 (1%, 5ML/KG) | 600.0±5.77 | 296.7±2.108 | 9.833±0.166 |
| 2 | Ethinyl estradiol | 1± g/rat/day | 1810±7.30*** | 645±4.282*** | 17.58±0.153*** |
| 3 | HECLR | 150 | 648 ± 3.07** | 316±1.667* | 9.833±0.166 |
| 4 | HECLR | 200 | 716.7±9.54** | 333.3±1.054** | 11.17±0.166** |
| 5 | HECLR | 250 | 1008±10.14*** | 523.3±6.146*** | 14.83±0.166*** |
| 6 | HECLR | 300 | 780±5.77** | 340±2.582** | 12.58±0.083** |

Physiology was also studied during anti-implantation and early abortifacient activity on the same group of experimental animals. Various parameters underwent changes were measured with the help of vernier callipars scale and also the height of epithelial cells. When they were observed they were found to be as follows:

1. Diameter of uterus increased with increasing dose till 250 mg/kg and then decreased on further increase in dose.
2. Thickness of endometrium increased with increasing dose till 250 mg/kg and then decreased on further increase in dose. and
3. Height of epithelial cells did not show any significant changes

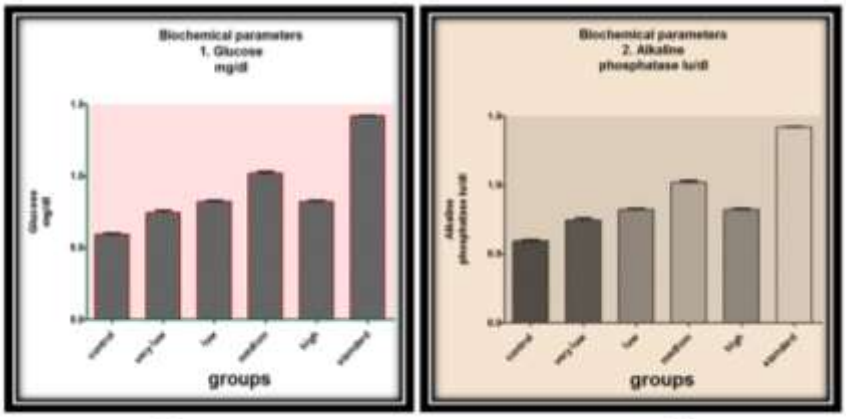


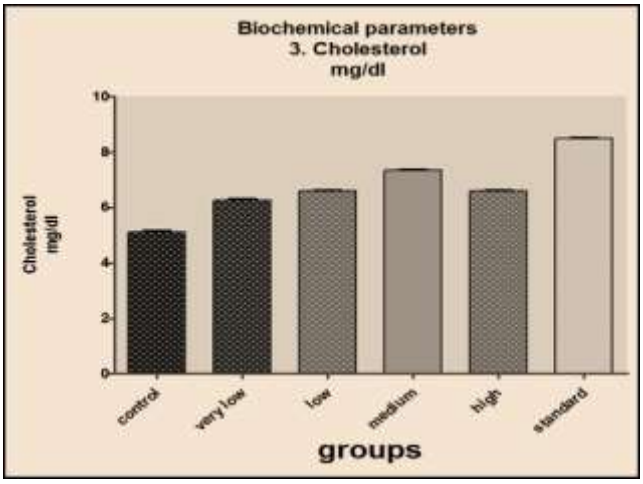

**Graph 8: Graph showing Micrometric changes in the uterus due to administration of various concentrations of extracts of *curcuma longa* rhizomes.**

**Table 7: Biochemical changes in the uterus due to administration of various extracts of *curcuma longa* rhizomes *curcuma longa* rhizomes.**

| S.No. | Treatment Extracts / Drugs | Dose (mg/kg) | Glucose mg/dl | Alkaline Phosphatase Iu/dl | Cholesterol mg/dl |
|---|---|---|---|---|---|
| 1 | Control (Vehicle) | Tween 80 (1%, 5 ml/kg) | 0.595 ±0.007 | 0.458 ±0.014 | 5.10 ±0.081 |
| 2 | Ethinyl estradiol (Estradial valerate) | 1 ±g/rat/day | 1.418±0.008*** | 0.933±0.013*** | 8.498±0.010*** |
| 3 | Hydro ethanolic extract | 150 mg | 0.746 ± 0.016** | 0.546 ±0.019** | 6.267 ±0.042** |
| 5 | Hydro ethanolic Extract | 200 mg | 0.823±0.009*** | 0.60 ±0.005*** | 6.60 ±0.036*** |
| 6 | extract ethanolic | 250 mg | 1.020±0.012*** | 0.706±0.005*** | 7.333 ±0.033*** |
| 7 | Hydro ethanolic extract | 300 mg | 0.823±0.009*** | 0.60 ±0.005*** | 6.60 ±0.036*** |




(A) (B)




**(C)**

**Graph 9: Graphs a, b and c showing Biochemical changes in the uterus due to administration of various extracts of *curcuma longa* rhizomes.**

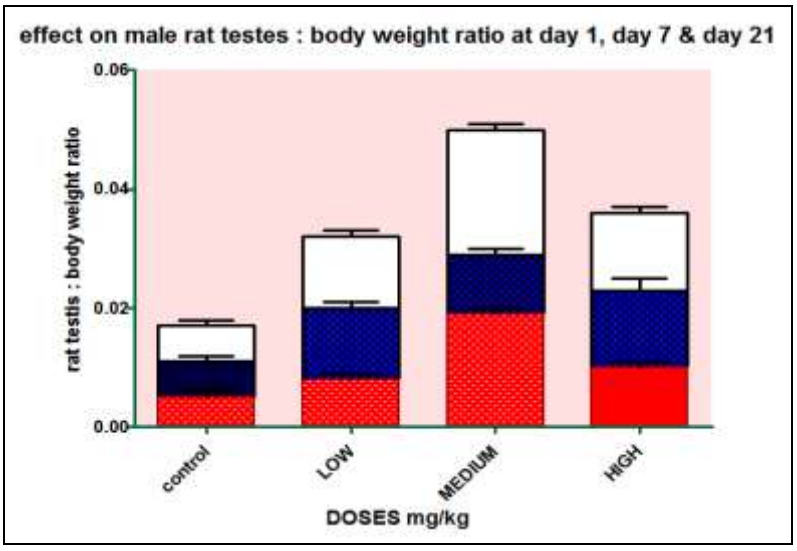
Enzyme study showed significant changes. Enzymes like alkaline phosphatase and other biochemical parameters like glucose and cholesterol level were determined after different doses of treatment with drug extract.

**Studies on male reproductive parameters**

**Table 8: Effect of hydro-ethanolic extract of curcuma longa on male rat testes–body weight ratio.**

| Test samples | Doses (mg/kg) (body weight) | Days (after-administration) | | |
|---|---|---|---|---|
| | | 1 | 7 | 21 |
| Control | 1ml | 0.005±0.001 | 0.006±0.001 | 0.006±0.001 |
| Hydro-ethanolic extract | 200 | 0.008±0.001 * | 0.012±0.001 * | 0.012±0.001 * |
| | 250 | 0.019±0.001 * | 0.010±0.001 * | 0.021±0.001 * |
| | 300 | 0.010±0.001 * | 0.013±0.002 * | 0.013±0.002 * |

*n*=5±S.D.; values carrying asterisk (*) down the column for each day are significantly different from their respective control value ($P < 0.05$)



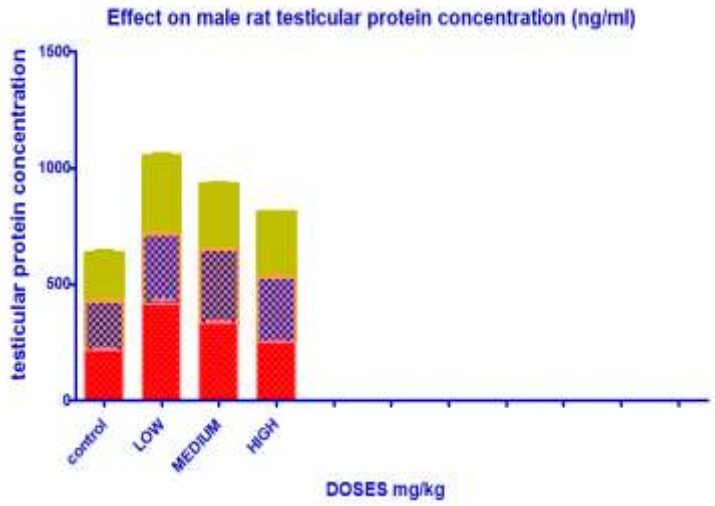

**Graph 10: Graph showing effect of *curcuma-longa* hydro-ethanolic extract on male parameters.**

Administration of hydro-alcoholic extract of *curcuma longa* rhizomes at the doses of 200 and 250 mg/kg body weight resulted in significant increase ($P < 0.05$) in testicular body weight and a declination at dose of 300 mg/kg ratio right from the first day up till the end of the experimental period. By the end of the experimental period, the testes–body weight ratio had increased to about 2.6-fold in the group administrated 250 mg/kg of dose.

**Table 9: Effect of administration of hydro-ethanolic extract of *curcuma longa* on male rat testicular protein concentration (ng/ml).**

| Test samples | Doses (mg/kg body weight) | Days (after-administration) | | |
|---|---|---|---|---|
| | | 1 | 7 | 21 |
| Control | 1ml | 216.00±10.00 | 210.00±10.00 | 211.00±10.00 |
| Hydro-ethanolic extract | 200 | 416.32±19.60* | 299.50±10.00* | 338.40±10.00* |
| | 250 | 331.30±5.00* | 319.00±4.02* | 285.00±3.17* |
| | 300 | 251.00±10.00* | 277.00±10.00* | 284.52±2.30* |

*n*=5±S.D.; values carrying asterisk (*) down the column for each day are significantly different from their respective control value ($P < 0.05$).




**Graph 11: graph showing effect of *curcuma-longa* hydro-ethanolic extract on male testicular protein concentration.**

**Table 10: Effect of administration of extract of *curcuma longa* rhizome on male rat testicular glycogen concentration (mg/g)**

| Test samples | Doses (mg/kg body weight) | Days (after-administration) | | |
|---|---|---|---|---|
| | | 1 | 7 | 21 |
| Control | 1ml | 2.30±0.02 | 2.28±0.05 | 2.32±0.00 |
| Hydro-ethanolic extract | 200 | 2.44±0.01* | 2.84±0.03* | 2.97±0.02* |
| | 250 | 2.78±0.03* | 2.99±0.04* | 3.26±0.05* |
| | 300 | 2.69±0.03* | 2.97±0.03* | 3.14±0.06* |

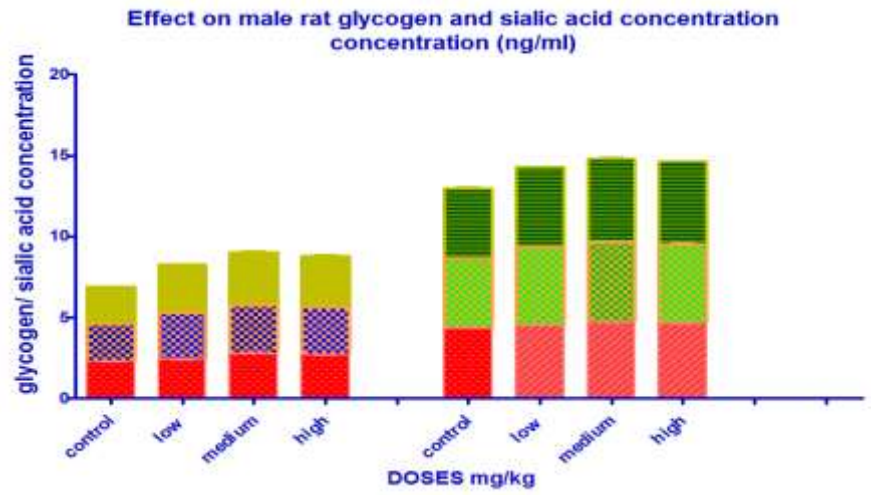
*n*=5±S.D.; values carrying asterisk (*) down the column for each day are significantly different from their respective control value ($P < 0.05$).

**Table 11: Effect of administration of extract of *curcuma longa* rhizome on male rat testicular sialic acid concentration (mg/g)**

| Test samples | Doses (mg/kg body weight) | Days (after-administration) | | |
|---|---|---|---|---|
| | | 1 | 7 | 21 |
| Control | 1ml | 4.32±0.03 | 4.33±0.04 | 4.31±0.04 |
| Hydro-ethanolic extract | 200 | 4.51±0.02* | 4.84±0.01* | 4.91±0.03* |
| | 250 | 4.69±0.03* | 4.94±0.02* | 5.15±0.06* |
| | 300 | 4.66±0.01 * | 4.88±0.05* | 5.08±0.04* |

*n*=5±S.D.; values carrying asterisk (*) down the column for each day are significantly different from their respective control value ($P < 0.05$

As amount of rat testicular protein concentration, testicular glycogen concentration and testicular sialic concentration are biochemical parameters which support the presence of androgenic activity in the drug extract, were found to increased with the dose increment till to 250 mg/kg.




**Graph 12: graph showing effect of *curcuma-longa* hydro-ethanolic extract on male rat glycogen and sialic acid concentration.**

**Table 12: Effect of administration of extract of *curcuma longa* rhizome on male rat testicular testosterone concentration (ng/ml).**

| Test samples | Doses (mg/kg body weight) | Days (after-administration) | | |
|---|---|---|---|---|
| | | 1 | 7 | 21 |
| Control | 1ml | 3.37±0.02 | 3.35±0.03 | 3.38±0.02 |
| Hydro-ethanolic extract | 200 | 3.42±0.03 | 3.69±0.01* | 3.80±0.02* |
| | 250 | 3.60±0.03* | 3.85±0.05* | 5.38±0.05* |
| | 300 | 3.59±0.01* | 3.77±0.03* | 4.10±0.06* |

*n*=5±S.D.; values carrying asterisk (*) down the column for each day are significantly different from their respective control value ($P < 0.05$).

**Table 13: Effect of administration of extract of *curcuma longa* rhizome on male rat serum luteinizing hormone concentration (mg/ml).**

| Test samples | Doses (mg/kg body weight) | Days(after-administration) | | |
|---|---|---|---|---|
| | | 1 | 7 | 21 |
| **Control** | **1mL** | **1.27±0.04** | **1.30±0.02** | **1.29±0.02** |
| **Hydro-ethanolic extract** | **200** | **1.37±0.01*** | **1.51±0.02*** | **1.56±0.01*** |
| | **250** | **1.60±0.02*** | **1.91±0.02*** | **2.14±0.04*** |
| | **300** | **1.48±0.01*** | **1.67±0.02*** | **1.97±0.03*** |

*n*=5±S.D.; values carrying asterisk (*) down the column for each day are significantly different from their respective control value ($P < 0.05$).

Effect of leutinizing hormone (LH) was found to be directly dependent on increasing dose of treatment of drug extract but further when it was found to increase by 50mg/kg more suddenly amount started decreasing, of LH.

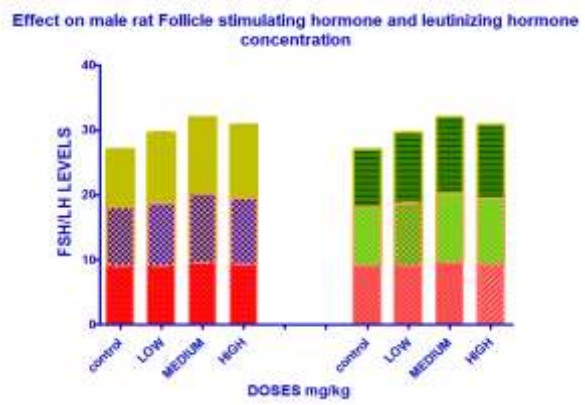
**Table 14: Effect of administration of hydro-ethanolic extract of *curcuma longa* rhizome on male rat serum follicle stimulating hormone concentration (ng/ml).**

| Test samples | Doses (mg/kg body weight) | Days(after-administration) | | |
|---|---|---|---|---|
| | | 1 | 7 | 21 |
| **Control** | **1ml** | **9.04±0.04** | **9.05±0.02** | **9.01±0.03** |
| **Hydro-ethanolic extract** | **200** | **9.06±0.03** | **9.71±0.01*** | **10.88±0.06*** |
| | **250** | **9.53±0.01*** | **10.71±0.02*** | **11.75±0.04*** |
| | **300** | **9.30±0.04*** | **10.21±0.06*** | **11.31±0.07*** |

*n*=5±S.D.; values carrying asterisk (*) down the column for each day are significantly different from their respective control value ($P < 0.05$)

Now, watching for the above results which are the outcome of the experiments performed on male rats. They were found to change drastically. All the levels – FSH, LH and Progesterone levels were found be

increased with the treatment of dose at 200 and 250 mg/kg and, further when dose increased to 300 mg/kg these levels were found to decrease.




**Graph 13: Graph showing Effect of administration of extract of *curcuma longa* rhizome on male rat serum luteinizing hormone concentration (mg/ml).**

## RESULTS AND DISCUSSION

For studies on female reproductive system, all parameters like smear count and uterine weight were suggesting estrogenic activity of plant extract. 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 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 extract, showed stronger activity, 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. 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 feedback 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. Various parameters evaluated in this study are useful indices to assess the potentials of a plant as anti-implantation and estrogenic/anti-estrogenic agents as well as the presence of abortifacient activities and its mechanism of action. The increase in the postimplantation loss observed with the extract suggests anti-implantation effect and consistently emphasizes the abortifacient or fetal resorptive properties[17] of the HEECLR extract. The postcoital, anti-implanta-tion activity of the extract in the rats may account for themechanism of abortion by the extract and may explain thefolk reputation of the plant as an abortion promoter. The measurement of the concentration of several female reproductive hormones such as progesterone, FSH and LH, as well as human chorionic gonadotropin hormones in the body fluid plays a significant role in determining ovulation and characterizing luteal phase defects.[18] Quantitativedetermination of the hormone concentration in human serumcould also be used to diagnose an ectopic or failing pregnancy.[19] In the present study, specific hormones were assayed based on their roles in maintaining pregnancy, since it has been shown that a failing pregnancy could be correlated to the levels of these hormones[20]in body fluids. The reduction in the concentration of FSH is an indication of impairment in the reproductive cycle of the animals.[21] LH is required for continued development and normal function-ing of the corpora lutea. The significant reduction in the level of serum LH could be associated with the physiological process of luteolysis preceding parturition.[22] It could possibly be attributed to pregnancy failure resulting from a luteal phase that is not being maintained. The reduced level of the hormone may also be due to inactivation of lutenization of ovarian follicles[23], which could be responsible for the reduction in the concentration of

serum progesterone in this study. Progesterone, which is elevated during pregnancy, plays a major role in maintaining the condition and is an important factor in the implantation process during pregnancy. Therefore, luteolysis and the decrease in the blood levels of progesterone may contribute to abortion and anti-implantation activity of the rhizome extract in this study. Similarly, such decrease in the serum progesterone level in the extract-treated animals may be the consequence of impairment in the endometrial function which prevents the secretion of special protein needed to nourish an implanted fertilized egg. The reduction in the hormone as observed in this study is possibly one step that facilitates the onset of labor. Our findings in this study agree with that of Al-Dissi et al.[24] on the effect of Nula viscose leaf extract on abortion and implantation in rats. Many anti-fertility plant extracts are known to exhibit estrogenic activity.[25] Estrogen causes an increase in protein synthesis, uterine weight, water uptake and retention of fluid leading to ballooning of the uterus.[25] It may also induce uterotrophic changes, causing vagina opening, which is a qualitative measure of estrogen potency.[26] Estrogen is also known to increase the uterine content of glucose, cholesterol, glycogen and alkaline phosphatase activity, thereby producing alterations in the uterine milieu and creating hostile conditions in the uterus.[26] Although the weight of the uterus, uterine/body weight ratio, length of the right uterine horn and the uterine cholesterol concentration were not altered, the decrease in the activity of alkaline phosphatase and glucose concentration may result in an environment that is not conducive for the continued development of the fetus. The provoked vaginal opening could possibly indicate estrogenic activity of the extract. This may, however, contribute, at least in part, to the mechanism of abortion of the plant. The finding in this study contrasts that of Koneri et al.[26] where it was reported that the ethanolic extract at 100and 200mg/kg bodyweight produced no estrogenic effect on immature rats. 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 drug extract 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 hydro-ethnolic extract of the plant showed positive results for glycosides, alkaloids and it is known from previous studies of different authors[1,2] that alkaloids and glycosides have contraceptive properties. Alkaloids and glycosides mainly flavonoids[3], 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. Preliminary phytochemical screening showed that plant 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, isoflavon 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,[4] 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.[5] Alkaloid of plant origin also possesses a frank estrogenic activity, as reported earlier[6] which it may be recalled is found to be present in extract of the plant in present study. A typical estrogenic compound possesses ability to increase the uterine weight[7], 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 hydro-ethanolic extract of *Curcuma longa* are responsible to induce frank estrogenic activity, as proved by cornification and opening of vagina in immature treated rats. The plant product 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. The plants extract 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 hydro-ethanolic extract of plant as extract showed positive test for alkaloids. Watching for male reproductive parameters, the evaluation of biochemical parameters of testes–body weight ratio, concentrations of testicular secretory constituents like protein, cholesterol, sialic acid, glycogen, testos-terone, testicular gamma glutamyl transferase activity, serum luteinizing hormone and follicle stimulating hormone concen-trations can give useful information on the androgenic and anti-

androgenic potential of chemical compounds and plant extracts. The parameters can also be used to evaluate normal functioning capacity of the testes.[8,9;10,11,12] Organ–body weight ratio is an index of inflammation or cell constriction.[13] An increase in organ–body weight ratio may either indicate inflammation or an increase in the secretory ability of the organ while a reduction in the parameter may imply cellular constriction. Therefore, the increase in the testes–body weight ratio (Table 1) observed following the administration of the plant extract may be attributed to increased secretory activity of the testes which in this present study is supported by the increase in the concentrations of cholesterol, protein, and testosterone, and luteinizing hormone, follicle stimulating hormone, sialic acid and glycogen. It may also be due to enhanced production of testicu-lar androgen (which is the case in this study) possibly as a result of increase in the mass of Leydig cells.[13] Such increased secretory activity of the testes further buttressed the androgen releasing property of the plant extract. This pattern of increase in testes–body weight ratio agrees with the work of[14] following the administration of Zingiber officinale in male rats. Testicular proteins are one of the constituents that ensure the maturation of spermatozoa.[15] Increased weight and high protein concentration of the testes indicates enhancement of testicular growth as follicle stimulating hormone is also essential for protein synthesis in the gonads.[16] Alterations in the secretion and functions of these proteins which are maintained by androgens may impair sperm maturation. The observed increase in testicular protein (Table2) following extract administration may be the result of testosterone action.[17] Such increase in protein concentration may enhance sperm maturation which is an important component of androgenicity.

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