

Antifertility Activity of *Curcuma Pseudomontana* in Comparison with *Curcuma Longa*

Pramod Reddy Aloor¹, Naresh Reddy K^{1,2*}, Goverdhan P¹, Srinivas Reddy CH¹, Hariprasad M¹, Kiran Kumar P¹ and Alabadri Anil³

¹Department of Pharmacology, Vaagdevi College of Pharmacy, Warangal, Telangana, India

²Department of Regulatory Affairs, LifePharma FZE, Jebel Ali, Dubai, UAE

³Department of Pharmaceutical Analysis, Kakatiya University, Warangal, Telangana, India

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*For Correspondence

Naresh Reddy K, Department of Regulatory Affairs, LifePharma FZE, Jebel Ali, Dubai, UAE, Tel: +971551854191.

E-mail: nareshreddy5431@gmail.com

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ABSTRACT

Collection and authentication of *Curcuma pseudomontana* and *Curcuma longa*. The methanolic extraction of dried plant material is carried out by soxhalation process and preliminary chemical test of extract to identify the phytoconstituent. To evaluate the antifertility activity of *Curcuma pseudomontana* and *Curcuma longa* in male and female rats. Comparison of antifertility activity in *Curcuma pseudomontana* with *Curcuma longa*

INTRODUCTION

Population explosion is a leading cause of poverty and population in developing countries. Several potential approaches for infertility have been investigated over a long period. Till date, steroidal pills and injections, barrier methods, sterilization devices are available for contraception, but the changing life style and increasing population burden telling us that the ideal contraceptive is yet to be discovered. Abortion is most often viewed as a ready remedy for unwanted pregnancy. Worldwide 42 million abortions are estimated to take place annually of which 20 million are unsafe abortions resulting in 70,000 deaths and 5 million disabilities per year ^[1].

Need for Investigation

- By using the synthetic antifertility drugs there are many side effects like nausea, vomiting, irregular bleeding, breast tenderness, hair loss, and cardiovascular diseases may occur.
- Due to this many herbal plants are used now a day like *Azadiracta indica*, *Aloe vera*, *Carica papaya*, *Piper bettle*, and *Hibiscus rosasinensis*
- *Curcuma longa* has a proven antifertility activity and other plants belonging to zingiberacea family like *Curcuma aromatica*, *Curcuma australasica*, *Curcuma zedoria* have proven antifertility activity ^[2].
- We are interested in investigating the antifertility activity of the curcuma species *Curcuma pseudomontana*.
- The rhizome of the plant, turmeric from which the active principle curcumin is obtained.
- Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions.

- The main mechanism for antifertility activity is curcumin which inhibits 5 α reductase which converts testosterone to 5 α dihydrosterone, there by inhibiting the human sperm motility and has potential for the development of novel intra vaginal contraceptive ^[3].
- There have been no reported adverse effects of curcumin despite a chronic daily intake of 80-200 mg in some Asian countries ^[4].
- Antifertility activity of *Curcuma pseudomontana* was not so far documented on this plant.

Plant Profile

Curcuma pseudomontana Graham

Synonym: *Curcuma ranadei* Prain

Taxonomical classification

Genus: *Curcuma*

Species: *pseudomontana*

Family: Zingiberaceae

Vernacular names

Telugu: *Adavi pasupu*

English: Hill turmeric

Tamil: Kattu manjal

Hindi: Kachura

Hill turmeric

Herb: It is an erect herb, growing to 75 cm tall.

Tubers: Fleshy and white inside, aromatic.

Flowers: 2-4 in each fertile bract, bright yellow.

Leaves: 3-5, oblong, base acute, tip sharp, margin entire

Traditional Uses

The Savara tribes in the Eastern Ghats of Andhra Pradesh use tuber extracts to cure jaundice. Jatapu and Kaya tribes apply warm tuber paste to treat body swellings. Women of Jatapu and Savara tribes eat boiled tubers to increase lactation. Khand tribes apply tuber paste on the head for cooling effect (Medicinal and aromatic plants abstracts). Reported activities are leprosy, dysentery, cardiac disease, haemorrhages, dysuria, general debility ^[5].

MATERIALS AND METHODS

Plant Material

Dried rhizomes of *Curcuma Pseudomontana* procured from Prof. Dr. K. Madhava Chetty, Department of Botany, SVU University, Chittoor, Andhra Pradesh, India.

Preparation of the Methanolic Extract

The rhizomes of *Curcuma pseudomontana* and *Curcuma longa* were shed dried at room temperature. The dried rhizomes are crushed by mechanical grinding. The powder was continued to soxhalation using methanol for 72 hrs at 65 °C. The concentrated extract was obtained by evaporating the solvent. The percentage yield of the extract is calculated and is found to be 8% w/w and kept in air tight container until use.

Animals

Albino Wistar rat's male and female about 150-180 gm were obtained from Teena Biolabs Pvt Ltd, Reg no. 177/99/CPCSEA Hyderabad. Animals are divided into five groups for male and five groups of females.

Phytochemical Studies

Various tests for analysis of different constituents of plant material such as fatty acids, alkaloids, carbohydrates and glycosides, phytosterols, steroids, tannins, phenolic compounds and proteins were performed.

Sperm Motility

Cauda epididymal sperm motility one hundred milligram of each tissue was minced in 1 ml of physiological saline. For sperm motility, one drop of evenly mixed sample was applied to a glass slide under a cover glass. The slides on which the sperm cells were counted were warmed to 37 °C until the time of the analysis. The analysis was carried out at room temperature using one epididymis of each rat. The percentage of sperm motility was calculated using the number of live sperm cells over the total number of sperm cells (both motile and nonmotile), from two samples from one epididymis of each rat. All sperm cells that were not moving at all were considered to be nonmotile, while the rest, which displayed some movement, were considered to be motile (Table 1) (Figure 1) [6].

Sperm Count

This was achieved using the new improved Neubauer’s counting chamber (Haemocytometer). The epididymal fluid was diluted with normal saline by adding 0.9 ml to 0.1 ml of the crushed epididymis. The counting chamber was next charged with a cover slip until a rainbow picture was seen at the edges. This chamber was then filled with sperm fluid and placed under a microscope using an adjustable light source. The ruled part was then focused, and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was then calculated and multiplied by 10⁶ and expressed as (X) × 10⁶/ml, where X is the number of sperm in a 16-celled square (Table 1) (Figure 2) [6].

Body Weight and Reproductive Organ Weights

The body weight of the animals were recorded before and after the treatment with the drug. After recording the final body weight at the end of treatment schedule, animals were sacrificed by decapitation. The testis and epididymis were dissected out, blotted free of blood and weighed. The weight is compared with that of the control (Figures 3 and 4) [7].

Biochemical Estimations

In male rats the testis was kept at -20 °C until assayed for cholesterol. The testis was homogenized with ice-cold distilled water in a pre-cooled mortar and pestle to contain 10 mg/ml. The homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was used for the estimation of Cholesterol (pNPP Kinetic Method) [8]. In female rats the ovary was kept at -20 °C until assayed for cholesterol. The ovary was homogenized with ice-cold distilled water in a pre-cooled mortar and pestle to contain 10 mg/ml. The homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was used for the estimation of cholesterol content using diagnostic kits (Figure 5) (Table 2) [9].

Anti Implantation Activity

Adult female virgin rats with normal estrous cycle were caged with males of proven fertility in the ratio 2:1 and examined for the evidence of copulation. Extracts were administered for 20 days. On the 20th day of pregnancy laproctomy will be performed. The animals will be sacrificed and the number of implantation sites will be determined.

Anti implantation and abortifacient activities will be calculated [10-12].

Anti implantation activity=No. Implants in control-No. of implants in test group × 100/Total No. of implants in control group

RESULTS AND DISCUSSION

Statistical Analysis

Comparisons are done by one-way ANOVA using Dunnet’s test. Comparisons are done between control group and the remaining groups. All the values are expressed in Mean ± SD. P-value ***p<0.001, **p<0.01, *p<0.05, ns-non-significant.

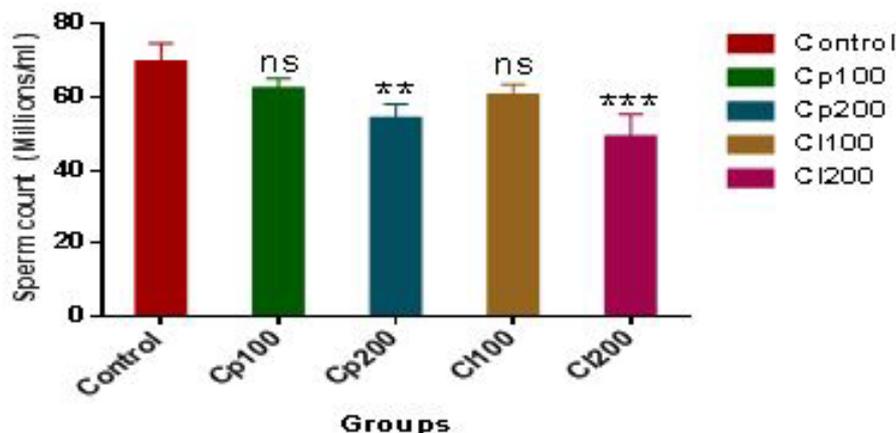


Figure 1. Effect of methanolic extract of *Curcuma pseudomontana* and *Curcuma longa* on sperm count.

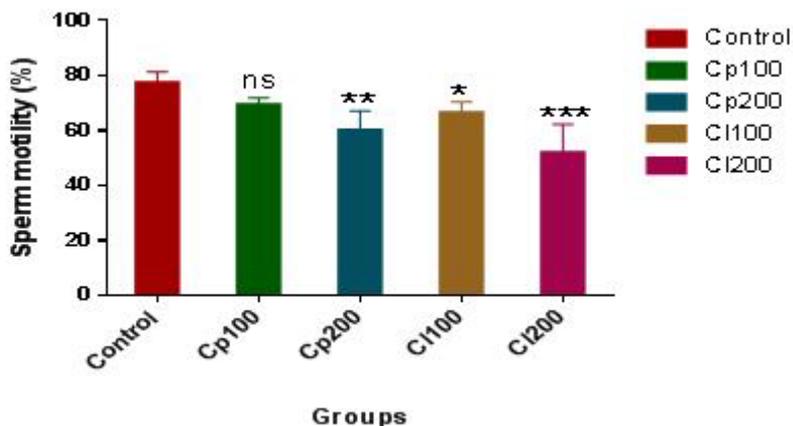


Figure 2. Effect of methanolic extract of *Curcuma pseudomontana* and *Curcuma longa* on sperm motility.

Table 1. Sperm count and sperm motility.

Groups	Sperm count (millions/ml)	Sperm motility (%)
Control	69.50 ± 5.19	77.25 ± 4.03
<i>C. pseudomontana</i>	62.25 ± 2.75 ^{ns}	69.25 ± 2.62 ^{ns}
<i>C. pseudomontana</i>	54.25 ± 4.99 ^{**}	60.50 ± 7.32 ^{**}
<i>C. longa</i>	60.50 ± 3.00 ^{ns}	66.25 ± 4.11 [*]
<i>C. longa</i>	49.00 ± 6.37 ^{***}	51.75 ± 6.56 ^{***}

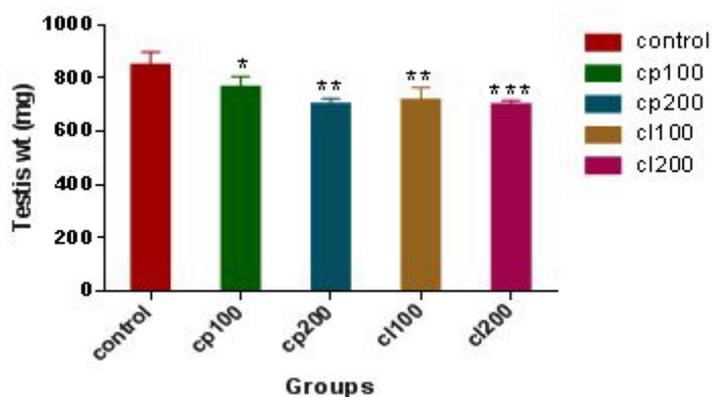


Figure 3. Effect of methanolic extract of *Curcuma pseudomontana* and *Curcuma longa* on testis weight.

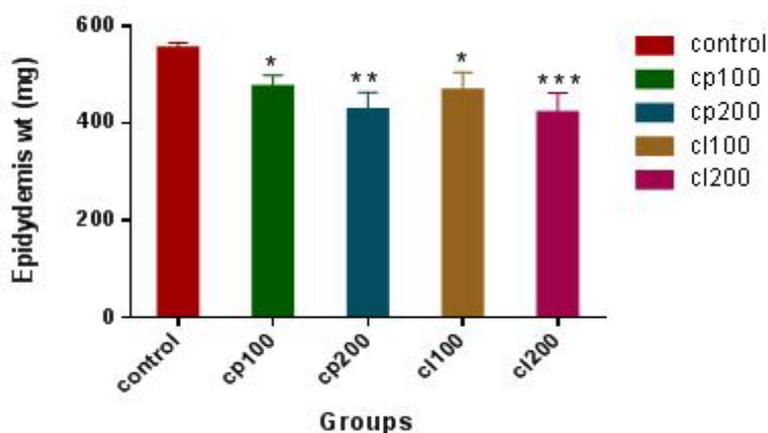


Figure 4. Effect of methanolic extract of *Curcuma pseudomontana* and *Curcuma longa* on epididymis weight.

Table 2. Biochemical estimations.

Groups	Cholesterol (mg%)
Control	118.69 ± 12.34
<i>C. pseudomontana</i> (100 mg/kg)	105.44 ± 5.67*
<i>C. pseudomontana</i> (200 mg/kg)	82.95 ± 2.40**
<i>C. longa</i> (100 mg/kg)	100.23 ± 5.03*
<i>C. longa</i> (200 mg/kg)	73.53 ± 4.16***

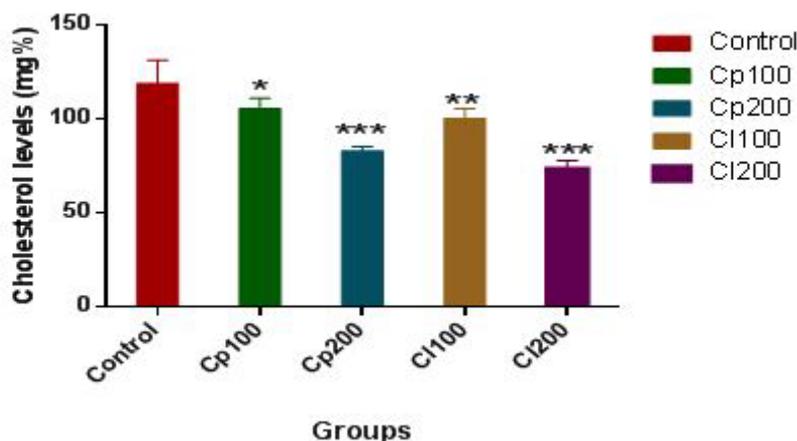


Figure 5. Effect of methanolic extract of *Curcuma pseudomontana* and *Curcuma longa* on cholesterol levels.

CONCLUSION

We are concluded that methanolic extract of *Curcuma longa* and *Curcuma pseudomontana* showed antifertility activity. But when compared to both *Curcuma longa* and *Curcuma pseudomontana*, *Curcuma longa* is shown more significant. In spermatogenic activity there is no significance at lower dose 100 mg/kg bw of *Curcuma pseudomontana* compared to higher dose 200 mg/kg bw of *Curcuma pseudomontana* and *Curcuma longa*. The antiimplantation and Abortifacient activity also showed more significance with curcuma longa 200 mg/kg bw when compared to other treatment groups.

By using the synthetic antifertility drugs there are many side effects like nausea, vomiting, irregular bleeding, breast tenderness, hair loss and cardiovascular diseases may occur but by using the herbal medicines there are no side effects for the long-term use also. Further studies should be necessary for isolation and characterization of active principles of the extracts responsible for the activity.

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