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ANTI-FERTILITY ACTIVITY OF CURCUMA LONGA LEAVES EXTRACTS IN NORMAL ALBINO RATS

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ABSTRACT

Various plants have been tested for their antifertility activity in lab animals. Various extracts of these plants were used on albino rats and other animals, to detect their mechanism of action and, henceforth, to isolate the active principle. The basic principle is to effectively compare the observations in test and control groups, after successful mating of adult female albino rats of proven fertility with male albino rats. Modern scientific research has confirmed anti-fertility activity in some of the herbs tested. Though, herbal contraception may never reach the level of contraception protection as the pills, varied effective herbal solutions have been found in recent years. In recent past a number of plants have been identified and evaluation of extracts and active principles from different parts of plant like seeds, roots, leaves, flower, stem or stem bark have been done by researcher.Hence present study was adopted to test antifertility activity of leaves of *curcuma longa* using albino rats. The evaluation of antifertility activity was done with ethanol with soxhlation followed by steam distillation to perform phytochemical screening.

INTRODUCTION

With the rapid increase in human population over the past three centuries, concerns have raised that the planet may not be able to sustain present or larger numbers of inhabitants. The Inter Academy Panel Statement on Population Growth has stated that many environmental problems, such as rising levels of atmospheric carbon dioxide, global warming, and pollution,

are aggravated by the population expansion[1]. Other problems associated with overpopulation include the increased demand for resources such as fresh water and food, starvation and malnutrition, consumption of natural resources faster than the rate of regeneration (such as fossil fuels), and a deterioration in living conditions. One option is to focus on education about overpopulation, family planning, and birth control methods, and to make birth-control devices like male/femalecondoms, pills and intrauterine devices easily available[2]. Worldwide, nearly 40% of pregnancies are unintended (some 80 million unintended pregnancies each year).In the developing world, some 514,000 women die annually of complications from pregnancy and abortion, with 86% of these deaths occurring in the sub-Saharan Africa region and South Asia[3]. Antifertility drugs inhibit ovulation and fertilization by showing Anti-implantation, Abortifacient, Anti-gonadotropic,Spermicidal, Inhibition of fusion of sperm & ovum, Decreased sperm count and sperm motility, Anti-androgenic activity. They are an effective and safe alternative to oral contraceptive pills. They will be the easiest method for achieving contraception in near future, with possibility of minimum undesirable effects[4]. Extensive research work and consequent statistical analysis, done on variety of plant extracts for years now, have shown how these antifertility drugs will change the face of contraception in modern world. In recent past a number of plants have been identified and evaluation of extracts and active principles from different parts of plant like seeds, roots, leaves, flower, stem or stem bark have been done by researcher[5].

BOTANICAL DESCRIPTION

The turmeric plant (*Curcuma longa*) is a perennial herb with aromatic rhizomes that are deep yellow in colour. Widely distributed across tropical and subtropical regions, it is predominantly cultivated in India, Southeast Asia, and parts of South America. The plant flourishes in warm climates, especially in well-drained soils, and is usually propagated through its underground rhizomes. Cultivation practices involve regular irrigation and care to ensure optimal yield [6, 7].

PHYTOCHEMISTRY

C.longa contains carbohydrates, fibre, certain proteins and lipids (no cholesterol), vitamin C, pyridoxine, magnesium, phosphorus, potassium, and calcium, which makes it a nutritionally rich natural food ingredient. It is found to contain over 235 phytoconstituents, the majority of which are polyphenols and terpenoids. Curcuminoids are made up of 80% curcumin and are the most common polyphenols [6]. There are sesquiterpenes, monoterpenes, diarylheptanoids

and diarylheptanoids, phenolics, diterpenes, sterols, triterpenoids, alkaloids, contained in it.Phenolic diketone curcumin provides yellow colour, and consists of curcumin I (94%), curcumin II (6%), and curcumin III (0.3%). Protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%), and moisture (13.1%) are reported [8].

PREPARATION OF THE PLANT EXTRACT

Collection & Drying

Fresh turmeric leaves were collected from Kolkata West Bengal and air-dried under shade for 20days.

Soxhlation and Distillation

After mechanical powdering of the dried leaves, finely powdered feed was produced for the soxhlation. Then the powder was subjected to hot soxhlation continuous percolation process using pure ethanol as solvent, maintaining temperature 100^0c-120^0c for 15days.The extract obtained was distilled for 1day until a sticky, semisolid, dark extract of the leaves were obtained. This is the pure extract,without the solvent. Total weight of crude drug taken was 56.5 gm andvolume of ethanol(solvent) used was.290 ml.Different phytochemicaltestson the ethanolic extract of *Curcuma Longa* leaves (obtained after soxhlation and distillation) were performed.

SEPARATION OF ACTIVE PRINCIPLES

Thin layer chromatography

TLC plates were prepared using silica gel G and activated by heating at 100 degree Celsius.Various plates were run for detection of alkaloids and flavonoids.Most suitable solvent for separation of flavonoids was selected as:Ethyl acetate: formic acid: glacial acetic acid: distilled water (100:11:11:27)

Column chromatography

After the most suitable solvent for separation of flavonoids was selected as: Ethyl acetate: formic acid: glacial acetic acid: distilled water (100:11:11:27), the column was set up using the selected solvent system. Reagents used- ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27).Weight of ethanolic extract of turmeric leaves taken = 5.5; For 100 ml

of slurry preparation: Silica gel G used=192 gm; Ethyl acetate = 67.11 ml; Formic acid= 7.38 ml; Glacial acetic acid = 7.38 ml; Water =18.12 ml (rest to make up 100); No of times taken = 3.The slurry was covered using thin layer of cotton plug above, and a solution of the extract was put above it to run the entire length of the column. Weight of plant extract taken= 0.6 gm in 20 ml of solvent system (Ethyl acetate =13.42 ml; Formic acid =1.47ml; Glacial acetic acid =1.47 meltwater = rest to make 20ml).This column was run for 48hrs, until the extract layer was seen to run down to the end of the column. After this, the liquid fractions were collected from the column at an interval of 5mins. 10 equal volumes of fractions were collected, until the fractions became almost colourless.

UV-VIS SPECTOPHOTOMETRICAL ANALYSIS OF FRACTIONS OBTAINED FROMTHE COLUMN CHROMATOGRAPHY

The fractions were taken as sample and the absorbance were observed using the solvent system as blank solution (Ethyl acetate: formic acid: glacial acetic acid: distilled water (100:11:11:27)) at 400nm.10ml of mixture of ethyl acetate: formic acid: glacial acetic acid: distilled water (in the ratio 100:11:11:27). Wavelength =400nm and taking readings.

Table no- 1. List of fraction number and absorbance

Fraction number	***Absorbance***
1	2.0496
2	4.0810
3	4.1155
4	4.2708
5	4.2025
6	1.1670
7	4.1826
8	4.4616
9	4.2635
10	1.7521

Now on fraction no 2 3 4 5 7 8 9, the following tests were performed: Now lead acetate test for flavonoids is done on fraction no 2 3 4 5 7 8 9. Yellow ppt is found in the entire fraction. Thus, we were confirmed that Flavonoids is present in the extraction *Performing Shinoda test*

on fraction no 5 shows prominent result presence of flavonoid) by changing the colour from red to crimsongetting the confirmation of presence of flavonoids further studies were done.

STUDY OF THE EFFECT OF CURCUMA LONGA ON ESTRUS CYCLE OF FEMALE ALBINO RATS

Theoestrus cycle is a cascade of hormonal and behavioural events.

- The total cycle length 4-5 days.
- Model for reproductive studies.
- The cycle is roughly divided in 4 stages (Proestrus, Oestrus, Metestrus, Dioestrus).

MATERIALS AND METHODS

Curcuma longa was taken as test drugs for the present study. Drugs were administered orally to the animal at a dose as per body weight.

Animals: Female Albino rats (125-150 gm) were used for present study. The animals (six per cage) were administered under standard laboratory conditions (light period 12h/day and temperature 27±2°C) with access to food and water ad libitum.

Design Study*:* A factorial study design was planned. The animals were divided into 3 groups containing 6 animals in each group.Group 1: Control, treated with Tween-80,1% orally; Group 2: treated with Ethanolic extract of the crude drug (50 mg/kg body wt. orally); Group 3: treated with Ethanolic extract of the crude drug (100 mg/kg body wt. orally) [9-15].

STUDY THE EFFECT OF EXTRACT ON THE ESTROUS CYCLE

Colony breed female albino rat of Wister strain (125-150 gm) were maintained under controlled standard animal house condition.Vaginal smear from each rat were monitored daily, only rats with normal oestrous cycle were selected for experiment.To study the effect of fraction of pet ether and ethanolic extract on the oestrous cycle.The above selected animals were divided into 3 groups containing 6 animals in each group.The group I received vehicle only (Tween-80, 1%) and served as control, Group ii and Group iii received ethanolic extract at doses of 50 and 100 mg/kg body wt respectively.The treatment was given for 15 days to

cover 3 regular oestrouscycles.A vaginal smear from the experimental animals was observed every morning.

EVALUATION OF ANTI-FERTILITY ACTIVITY

Table no- 2.List of evaluation of anti-fertility activity of control

Control (no.)	*Proestrus*	*Estrus*	*Metestrus*	*Diestrus*
1	2	2	10	1
2	2	2	10	1
3	3	2	9	1
4	3	2	9	1
5	3	1	9	2
6	3	2	9	1
Total	16	11	56	7

Table no- 3.List of evaluation of anti-fertility activity of 50 mg extract

50 mg test (no)	*Proestrus*	*Estrus*	*Metestrus*	*Diestrus*
1	4	0	11	0
2	4	0	11	0
3	3	3	6	3
4	4	3	6	0
5	4	3		2
6	3	3	7	2
Total	22	12	47	7

Table no- 4.List of evaluation of anti-fertility activity of 100 mg extract

100 mg test (no.)	*Proestrus*	*Estrus*	*Metestrus*	*Diestrus*
1	0	0	13	2
2	1	2	9	3
3	0	3	6	6

4	1	3	7	4
5	2	2	6	5
6	1	1	8	5
Total	5	11	49	25

STATISTICAL ANALYSIS

The data were statistically analyzed and expressed as mean ±SEM. The Student t-test was used to determine significant difference between treated and control group[16-34].

Table no- 5. List of ethanolic extract on duration of different phases of oestrous cycle.

Group	***Treatment***	***Dose (mg/kg body weight)***	***Mean days of estrus±SE***	***Mean days of metestrus±SE***	***Mean days of diestrus±SE***	***Mean days of proestrus±SE***
I	Control	Tween 80, 1% orally	1.833±0.657	9.333±1.038	1.666±0	2.666±0.298
II	Ethanolic extract	50mg	2±0.666*****	7.8333±1.030*****	1.666±0*****	3.666±0.299*****
III	Ethanolic extract	100mg	1.833±0*****	8.166±1.096*****	4.166±0.621*****	0.833±0.377*****

SE = Standard Error; * = P<0.002; ** = P<0.01; *** =P<0.001; **** = P<0.5; ***** =P<0.1; ****** = Insignificant

RESULT

The results are divided in Table -II. Administration of ethanolic extract of *Curcuma longa* showed non-dose dependent effect on duration of Oestrus Cycle. The ethanolic extract of both doses (50mg/kg & 100mg/kg) increase the duration of Dioestrus& Proestrus Phases. A significant increase in Dioestrus& Proestrus Phases was observed in animals of both treated

groups when compared with control drug's experimental period. But experiment at both doses (50mg/kg & 100mg/kg) do not show any effect (insignificant) at Oestrus Phases & significant increase in Meta-oestrus Phases[35-45].

DISCUSSION

The oestrus Cycle in female involves many histological, physiological & biochemical changes into overies. During the oestrous cycle the maturation & ovulation of pre-ovulatory follicles takes place under the combined & balance influence of ovarian & extra ovarian hormones. Any imbalance in the hormones leads to irregularity in the function of ovary& irregular changes in duration of oestrous cycle.Oestrogen level are lower during estrous phases and gradually increases during dioestrus phases to reach peak at Proestrus Phase. The Progesterone hormone is low during oestrus phases and high during dioestrus phases and highest during prestrous phases. The increase in duration of oestrus,proestrus and dioestrus phase treated rats, further increase in LH & FSH level upon administration of extract.The oestrus cycle changes due to plant extract having flavonoid, glycoside, carbohydrate, alkaloids. Thus the plant havinganti-fertility Activity[46-49]**.**

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