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Evaluation of antifertility activity of *Limonia cranulata L.* in albino rats.

S. Manikandaselvi*, S. Geetha and E. Priya

PP. G Department of Biochemistry, STET Women's college, Mannargudi.

Abstract

Antifertility activity of different dosages of *Limonia cranulata L.* in albino rats was studied. The parameters used in the study include number of implants in individual, number of neonates, weight of neonates, pregnancy rate, cholesterol level in ovary and estradiol content of female rats, and number of sperm cell and testosterone from male rats. Administration of *L. cranulata L.* demonstrates implantation activity and reduced number of neonates, that is consistent with its use in folk medicine as an contraceptive agent.

Keywords : Antifertility, *Limonia cranulata* , neonates.

INTRODUCTION

Population means the number of inhabitants in a specific territory. The United Nations estimated the world population as 5.385 billion in the middle of 1991(Bingel,1973). During 1980-1990 the average rate of increase was 1.7%. India will cross seven billion marks by 2022 (Barbara, 1992). Asia has 58.8% of the world population with a growth rate of 2.2%. India comes second with 16% of the population after China, which has the largest population in the world. Food production is increasing in an arithmetic progression, while the population is increasing in geometric progression. Compared to natural and human resource of the country, India is definitely over populated. To control the menace of population explosion, many nations have enmarked various programmes of family welfare.This has brought down the rate of population to some extent (Khanna,1968).

There are manifold cause for over population .India is known for early marriages, this gives longer span for reproductive activity .The other contributing factors for over population are tropical, climate, total beliefs, ignorance , illiteracy, lack of respect for women, lack of recreational facilities, scarcity of technical advices ,absence of welfare schemes and a vertical decline of death rate (Riar,1991). The uncontrolled growth of population results in creation of various fundamental problems like unemployment, inadequate civic facilities, overcrowding of urban areas, low per capital income, starvation, sub human conditions of life and increase in crime rate.

Hence the fertility control has become most important and urgent mainstay of all biomedical and biosocial problems facing the mankind (Kasturi, 2002). The need for evolving more acceptable and more effective means of contraception, for both male and female, with nil or minimum side effects is more actually felt now, than ever before. India has rich heritage of use of medicinal plants for fertility controls. In this context, it will be appropriate to locate indigenous plants that are used as oral contraceptives by tribes and other sections. Such plants are even recommended in folk medicines and Ayurvedic medicines from very ancient times. Several scientific papers have already been published related to use of medicinal plants for fertility control. But, still many more medicinal plants are left uninvestigated. Owing to this fact, many scientists are presently engaged in the search for a safe,acceptable both by male and female , effective, easily administrable, reversible, cheep and non-steroidal antifertility agents from the extracts of plants, commonly grown in India and other parts of the world. In the present study, *L. cranulata* L. (*Rutaceae*) was used for the investigation of antifertility activity.

MATERIALS AND METHODS

Experimental Animals

Albino rats (150-200g) of either sex of 6–8 weeks age were selected for this study. The animals were kept in clean and dry plastic cages, with 12h : 12h light–dark cycle at 25 ± 2°C temperature and 45 – 55 % relative humidity. The animals were fed with standard pellet diet and water was given *ad libitum*.

Preparation of herbal drug

The plant, *Limonia cranulata* was collected from herbal garden of S.T.E.T Women's college, Mannargudi. Collected plant was carefully examined and then identified with help of regional flora. Specimen was further confirmed with reference to herbarium sheets available in the Rapinat Herbarium, St.Joseph college, Thiruchirappalli.

The leaves and fruits were dried in shade and subjected to pulverization to get fine powder. The collected parts of this plant were processed by Murugesha Mudaliyar method and filtrated through "Vasthrakaya" method.

*Corresponding author :
email: selvithinagar@gmail.com

Herbal Drug Administration

Different concentrations (50,100,150,200,250mg/kg/day) of this herb were administered through oral route. Plant fine powders were mixed thoroughly using distilled water. Before herbal drug administration, the animals were allowed to stand for 2h to enhance the intestinal absorption of drug. Experimental duration was 21 days.

Experimental protocol

Rats were used for the test after checking their proven fertility through a preliminary mating system with selected rats. They were housed under standard condition of temperature (24±10^{0}c), relative humidity 65±10%, light and dark cycle14:10h and fed with standard pellet food (Tamil Nadu Vertinary college, Veppari,Chennai) and water *ad libitum*.

Experimental design

Group I: Rats were kept as control administered only with standard diet.

Group II: Rat was administered with 50mg powder of *L. cranulata* once daily and administered for 21 days.

Group III: Rat was administered with 100 mg powder of *L. cranulata* once daily and administered for 21 days.

Group IV: Rat was administered with150 mg powder of *L. cranulata* once daily and administered for 21 days

Group V: Rat was administered with 200 mg powder of *L. cranulata* once daily and administered for 21 days.

Group VI: Rat was administered with 250 mg powder of *L. cranulata* once daily and administered for 21 days.

On the 22nd day, all animals were sacrificed under an over dose of anesthesia .The serum was collected and the uterus were excised and washed in the ice-cold saline ,dried ,weighed ,cut into piece and immediately processed for biochemical estimations.

Acute toxicity study(Vishnu N.Thakare, 2009)

Acute toxicity study of the extract of *L. cranulata* was carried out in albino rats according to OECD guidelines. Extract at different doses upto 2000 mg/kg, p.o. was administered and the animals were observed for behavioural changes, any toxicity and mortality up to 48 h. There was no toxic reaction or mortality, and found to be safe. Based on acute toxicity result the dosages of 50 mg/kg to 250 mg/kg for antifertility evaluation were selected.

Fertility test (Koneri,2006**)**

The vaginal smear of the female rats was studied microscopically for oestrous cycle every morning at 8-9am. Only female rats with normal oestrous cycle were selected for the antifertility activity evaluation. Prior to mating, the females were isolated so as to rule out the pre –existing pregnancy. Female and male rats were housed in the ratio of 3:1 per cage and in the next morning the vaginal smears were checked to examine the presence of sperm.

Anti implantation activity

Female rats were kept with male rat of proven fertility in the ratio of 3:1. The female rats were examined in the following morning for evidence of copulation. The animals which showed thick clumps of spermatozoa in vaginal smear were separated from the male partner. All the animals were sacrificed under light ether anesthesia and laprotomy was performed to determine the number of implantation sites on both uteri horn and the number of corpora lutea on both ovaries. The fertility rate was calculated on the basis of percentage of implantation per number of corpora leutea (representing number of eggs ovulated).

Number and weight of neonates

Each individual rat of every group was under observation for the delivery of neonates up to the protocol periods. After the time of delivery, each neonate was weighed and recorded.

Percentage of pregnancy

I. Adult female rats were kept with male rats at the ratio of 3:1.

II. Males were mated with fertile female. As a result of matting they were pregnated and after 3rd week, they delivered young ones and female rats were counted.

III. Percentage of pregnancy

$$= \frac{\text{deliverded rats}}{\text{total number of rats}} \times 100\%$$

Estimation of cholesterol(Zak,1959**)**

Cholesterol is the precursor for the steroidogenesis of ovarian endocrine tissues. It is well known that for implantation exact equilibrium of estrogen and progesterone is essential, any disturbance in the level of these hormones causes infertility.

Total sperm count (Updhyay,1990**)**

The sperm count was made using hemocytometer. The number of squares counted depends on the average number of spermatozoa per square as described below.

<10spermatozoa/square-count 25 squares

10-40spermatozoa/square-count10 squares

< 40spermatozoa/square-count 15 squares. Then the values were corrected by the correction factor to obtain number of spermatozoa in million/ml present in the original sample.

Sex hormones (Estradiol & Testosterone) were estimated by the method of Hafez (2009).

RESULTS

In this experiment, different dosages of the powder of the *L. cranulata* L produced a significant reduction of fertility in male and female albino rats, which was carried out through the parameters such as number of implants in individual, number of neonates, weight of neonates and pregnancy rate, ovarian weight, cholesterol level in ovary and estradiol content of female rats and number of sperm cells and testosterone from male rats.

The number and weight of neonates of the control animal was 9,12 and 10 and 5.34±0.05g . But this strength was gradually reduced due to the oral administration of test drug. There was complete absent in the group VI which had taken up 250mg of test drug (Table 1). Rate of pregnancy or infertility percentage calculation was antonyms for each other which was recorded from the experimental setup. The maximum amout of pregnancy (100%) rate was recorded from control 50 and 100mg/animal groups. Cent per cent of infertility was recorded from the VIth group which was administered with the 250mg/animal dosage.

Table 1: **Effect of various doses of herbal drugs on fertility in albino rats**

Groups	Dose mg/kg of body weight	No of implants in individual rat	No of Neonates delivered	Weight of Neonates	Rate of pregnancy percentage
Group I	Control	3/3	9,12,10	5.34± 0.05	100
Group II	50	3/3	10,7,6	5.36±0.07	100
Group III	100	3/3	5,8,5	5.29±0.04	100
Group IV	150	2/3	7,5	5.13±0.05	67
Group V	200	1/3	3,4	5.07±0.06	33.3
Group VI	250	0/3	-	-	-

Observation of cholesterol level of experimental groups, showed that higher amount of cholesterol was recorded from the control group of animal which was 0.43±0.02.The minimum amount of cholesterol was recorded from the higher concentration administered group VI (Table 2).

Table 2 : Effect of various doses of herbal drugs on fertility in albino rats

Groups	Dose mg/kg of body weight	Cholesterol level in Ovary(mg/100mg)
Group I	Control	0.43±0.02
Group II	50	0.42±0.03
Group III	100	0.41±0.05
Group IV	150	0.40±0.07
Group V	200	0.37±0.09
Group VI	250	0.33±50.07

The estradiol amout was maximum (38.4±3.04) in control groups. The higher amount of estradiol was gradually reduced from higher concentration of drug administered groups (Table 3). Number of sperm cell counts and testosterone amout was generally maximum in the control and minimum in drug administered groups. The plants, 'powder effect was significantly exhibited in experimental groups. Sperm cells count was gradually decreased in control to higher concentration groups which was $10^{-7}\times10^{6}$.The amount of testosterone range was 688.45±53.37 to 532.73±43.29 which were recorded from the experimental models.

Table 3: Effect of various doses of herbal drugs on fertility in albino rats

Groups	Dose mg/kg	Estradiol ng/dl of body weight	No of Sperm cells $\times 10^6$	Testosterone ng/dl
Group I	Control	38.40±04	10	688.45±53.37
Group II	50	39.20±02	10	692.37±57.31
Group III	100	36.30±03	10	667.63±45.28
Group IV	150	33.50±05	9	643.57±46.43
Group V	200	31.10±07	9	611.65±43.37
Group VI	250	25.30±06	7	532.73±43.29

DISCUSSION

Herbal medicine is the oldest form of medicine in which any parts of the plants or whole plants are used for medicinal purposes (Blatter, 2001). The remarkable rate of implantation occurred in 250mg of *Limonia cranulata* L. administered rats 0/3. There was neonates in the group VI which had taken up 250mg of test drug. Rate of pregnancy was 0 from the VIth group which was administered with the 250mg. Minimum amount of weight 31.26±0.39 was recorded from the group VI.

Different glands and organs throughout the body produced hormones (Gbotolorun,2004).For example, the pancreas secretes the hormone insulin, whereas the ovaries secretes estrogens and progesterone. Other glands such as the pituitary and hypothalamus in the brain secrete hormones such as follicle stimulating hormone and luteinizing hormone that control as to how much estrogen and progesterone are produced by the ovaries (Hirshfield,1991).

The major female hormone and male hormones can be classified as estrogens or androgens. Both classes of male and female hormones are present in both males and females alike, but in vastly different amounts. Most men produce 6-8mg of the male hormone, testosterone (androgen) per day, compared to most female who produce 0.5mg of estrogen (Mukherjee, 1999). Female hormones, estrogens, are also present in both sexes, but in larger amounts in female.

The higher amount of estradiol was gradually reduced due to the herbal drug dosage . The amount of testosterone range was 688.45±53.37 to 532.7 ±43.29, which were recorded from the experimental models.This was changed by the plants containing chemical constituents. A new indole alkaloid, crenulatine, along with twenty known compounds, were isolated from the stems of *L. cranulata* L. (Okanlawon,1992). Those compounds include four alkaloids,four coumarins,two flavanones,three tetra nortriterpenoids, one triterpenoid,three steroids, two lignans and two aromatic compounds.

Number of sperm cell counts and testosterone amount was gradually increased from the control to minimum drug administered groups.The plant powder effect was significantly exhibited in experimental groups. Sperm cells count was gradually reduced from control to higher concentration groups which was $10^{-7}\times10^6$.

It is well known that for implantation exact equilibrium estrogen and androgen is essential, and any disturbance in the level of these hormone caused infertility (Vander schoot,1982).The minimum amount of cholesterol was recorded from the higher concentration administered group VI. Loss of implantation caused by *L. cranulata* L. could be due to antizygotic or blastocytotoxic activity(Mukherjee,1996). The result of the present study showed that administration of *L. cranulata L.* demonstrates anti implantation activity and reduced number of neonates that is consistent with its use in folk medicine as anti-contraceptive agent. Further detailed study using different animal species to establish its antifertility activity has been suggested.

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