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## ANTIFERTILITY ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *SEMECARPUS ANACARDIUM* FRUIT IN FEMALE ALBINO RATS

Y. Sushma<sup>\* 1</sup>, Gajanana Kulkarni<sup>1</sup> and Shilender Singh<sup>2</sup>

Department of Pharmacology<sup>1</sup>, Subbhaiah Institute of Medical Sciences and Research Centre, Shivamogga, Karnataka, 577201, India.

Department of Pharmacology<sup>2</sup>, Bidar Institute of Medical Sciences, Bidar, Karnataka, India.

### Key words:

*Semecarpus anacardium*,  
diestrus, estrous cycle,  
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### Correspondence to Author:

**Dr. Sushma Y.,**

D/o B S Yogendra Prasad, Prasad  
clinic, Rangenahalli post, Tarikere  
taluk, Chikamagalore district  
Karnataka, India.


**Email:** sushmaprasad04@gmail.com

**ABSTRACT:** *Semecarpus anacardium* plant derivatives has been used since antiquity, The fruit and nut extract of *Semecarpus anacardium* shows various activities like antiatherogenic, anti-inflammatory, antioxidant, antimicrobial, antireproductive, CNS stimulant, hypoglycemic, anticarcinogenic, antifertility effects. The present study is an attempt to explore the antiovarulatory effect of aqueous and ethanolic extracts of fruits of *Semecarpus anacardium* plant in female albino rats. **Objective:** To study the antiovarulatory activity of aqueous and ethanolic extracts of *Semecarpus Anacardium* fruit in female albino rats. **Materials and Methods:** Total of 54 Wistar female albino rats were selected weighing about 150 – 200 grams with atleast three regular estrous cycles were divided into 9 groups containing 6 rats each were administered aqueous and ethanolic extracts of *Semecarpus anacardium* fruit both were administered in doses of 200,400,600,800 mg/kg, b.wt for different group daily for 21 days and estrous cycle monitored for 30 days daily. Control group received vehicle (Tween 80 1%, p.o. daily) and the estrous cycle was monitored daily, doses for the study selected after acute toxicity studies. **Results:** The aqueous and ethanolic extracts of *Semecarpus anacardium* fruit found to possess highly significant ( $P < 0.05$ ) increasing Diestrus index in a dose dependent manner. **Conclusion:** Aqueous and ethanolic extracts of *Semecarpus anacardium* fruit has got reversible antiovarulatory effects on female albino rats.

**INTRODUCTION:** Much attempts have been made to search for female antifertility agents from the plant source<sup>1, 2</sup> *Semecarpus anacardium* belongs to family Anacardiaceae and is commonly known as Bhilawa in Hindi. The fruits of plants are largely used in Ayurvedic system of medicine for various ailments, particularly alimentary tract and certain dermatologic conditions. It has beneficial effect on heart, blood-pressure, respiration, cancer, neurological disorders<sup>3,5</sup>.

Population explosion is a serious problem throughout the world and also in India, It is an imminent hurdle for a country's development as the natural sources are limited. The population of India is multiplying in an alarming rate and has crossed one billion<sup>6</sup>. It affects all aspects of development especially, employment, education, housing, health care, sanitation and environment.

India forms only 2.4% of world land area and supporting about 16.87% of the world population. Scientists from different parts of the world are making serious efforts to solve the problems of population explosion<sup>7</sup>. Moreover major population of our country lives in villages and those people do not have approach to the modern methods of Family planning. Fertility regulation has therefore become the major concern of people of all walks of

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life. Although a variety of synthetic contraceptive agents are available, their use is associated with adverse effects like breast cancer, cervical cancer etc. Traditional sterilization method based on herbal medicines is used to control population growth rate including abortion at initial weeks, preventing conception or making the either member of the couple sterile<sup>8,9</sup>. Hence it is in need of hour for the development of a reversible infertility with minimal side effects without altering the libido from plant sources hence this study has been considered. Hence, an approach was pursued to identify new antifertility agents from natural sources. Much attempts have been made to study antifertility effects on male albino rats using *Semecarpus anacardium* plant but no attempts have been made on female albino rats

#### Review of Literature:

*Semecarpus Anacardium* is commonly known as 'Marking nut', 'Oriental cashew', 'Bhilawa' or 'Bhallataka', Is a moderate sized semi-deciduous tree with grey bark that exfoliates in small irregular flakes. The leaves are simple, alternate, obovate - oblong rounded at the apex, glabrous above pubescent below. The greenish fruits are ovoid to oblong drupes that has attached to a swollen, fleshy receptacle that sit below it and turns yellow when ripe. The fruits are useful in Leucoderma, Scaly skin, Allergic dermatitis, Poisonous bites, leprosy, Cough, Asthma, Dyspepsia<sup>10, 11</sup> Several plant products inhibits male and female fertility and may be developed into contraceptives. Only few plants have so far been investigated for anti-fertility activity. It is necessary to use biologically active botanical substances or fertility regulating agents of plant origin which are eco-friendly in nature<sup>12, 13</sup>. Hence we have aimed at finding a safe contraceptive with minimal side effects using fruit of *Semecarpus anacardium* plant. This plant is also proved to be having anti-spermatogenic effects and there are no satisfactory data showing its effect on female reproductive system hence it was decided to study the effect of *Semecarpus anacardium* fruit on female reproductive system in female albino rats.

**Objective:** To evaluate the antioviulatory effect of aqueous and ethanolic extracts of *Semecarpus Anacardium* fruit in female albino rats.

#### MATERIALS AND METHODS:

The study was approved by the Institutional Animal Ethical Committee, the work was done in the department of Pharmacology, BRIMS, Bidar, in the year 2012 month of January and February. The study was conducted in accordance to Good Laboratory practice (GLP), regulations of the WHO.

#### Plant Material:

The fruits of *Semecarpus anacardium* were collected from the local market, in the month of December 2011, It was Identified and authenticated by a plant taxonomist. The collected plant was washed thoroughly in tap water and dried in room temperature for 15 days, course powdered and were extracted with distilled water and ethanol by soxhlet apparatus; extracts were used to evaluate the antioviulatory activity in female albino rats by monitoring the estrous cycle.

#### Acute Oral Toxicity Study:

Acute toxicity study was carried out as per prescribed OECD guidelines. Prior to experimentation animals (n=6) were fasted overnight (water withheld for 3-4 h) and was orally administered with fixed extracts dose of 100, 200, 400 and 2000 mg/kg/body weight respectively by gavage. The dose was found tolerable as no death and other adverse effects was found up to the maximum administered doses

#### Experimental Animals:

Female albino rats (Wistar strain) weighing 150-200grams were used in the study were obtained from animal house, BRIMS, Bidar. The animals were housed in standard cages of 10x10.5x8 cm size, 6 per cage, in a controlled temperature (22° c), The animals were acclimatized for 10 days under standard husbandry conditions; room temperature (27+ or -3 degree C) relative humidity (65= or -10%) and 12 hours of light and dark cycle. They were allowed free access to standard dry pellet diet and water *ad libitum* under strict hygienic conditions. All the described procedure were reviewed and approved by the Institutional Animal Ethical Committee (Reg No: 1216/a/08/CPCSEA).

**Experimental Design:** The female reproductive cycle was monitored by obtaining vaginal smears;

the female rats which showed normal and regular estrous cycle for 21 days covering 4 estrous cycle were selected and grouped into 9 having 6 rats each. Group 1 acted as control receiving Tween 80 1% orally, Group 2,3,4,5 receiving 200,400,600,800mg/kg b.wt. of aqueous extract orally respectively, Group 6,7,8,9 receiving 200,400,600,800mg/kg b.wt. of ethanolic extract orally respectively. Extracts were administered daily for 21 days and estrous cycle monitored daily for 30 days.

### Estrous Cycle Evaluation:

Vaginal secretions were collected daily using 3ml plastic pipettes filled with normal saline between

9:00 and 10:00a.m and smears prepared on glass slides, unstained material observed under light microscope for cells and estrous cycle phase of each rat is noted. There are four phases in the estrous cycle, namely, a) diestrus phase as in fig a., shows few leucocytes and nucleated parabasal and intermediary cells, b) oestrus phase-as in fig b., smear consists entirely of keratinized superficial cells, c) pro-oestrus phase- as in fig c, smear is lighter in colour and cells are predominantly intermediary cells, leucocytes and parabasal cells are rare, d) metoestrus phase – as in fig d., smear shows the presence of flakes of keratinized cells and leucocytes in large number and few intermediary cells.

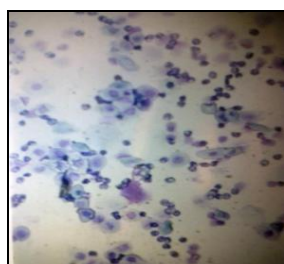
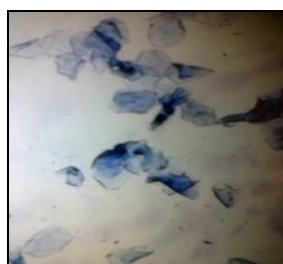
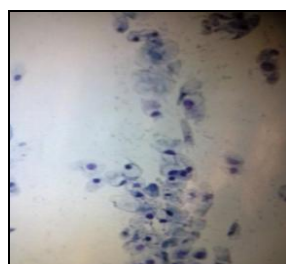


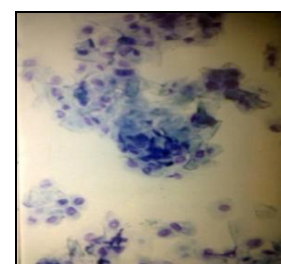
Fig: a) Diestrus phase



b) oestrus phase



c) metaestrus



d) proestrus

**RESULTS:** The rats exhibited prolonged diestrus phase of the estrous cycle with consequent temporary inhibition of the ovulation which was statistically significant in the group 5,7,8,9

compared with control ( $p < 0.05$ ), by ANOVA test as shown in the **Table 1**. The anovulatory effect was reversible on withdrawal of the extract.

**TABLE 1: SHOWING THE EFFECT OF SEMECARPUS ANACARDIUM FRUIT EXTRACTS ON DIFFERENT PHASES OF ESTROUS CYCLE**

Groups	Treatment In mg/kg b.wt.	Number of rats	Number of cycles	proestrus	estrous	metestrus	diestrus	Diestrus index
1	Control+0.9%NS	6	6.1±.21	4.7±.27	7.5±.23	5.6±.21	12.1±.42	40.10
2	Aq.ex.- 200	6	5.9±.25	4.63±.38	7.6±.25	5.3±.23	12.11±.43	40.36
3	Aq.ex-400	6	5.71±.18	5.02±.24	7.71±.28	4.86±.26	12.29±.42	40.78
4	Aq.ex-600	6	5.41±.19	3.5±.51	6.78±.25	5.1±.17	12.7±.35	42.86
5	Aq.ex-800	6	4.12±.19	2.8±.31	6.3±.31	4.6±.22	15.8±.51	52.66
6	Eth.ex-200	6	5.61±.28	4.1±.33	7.4±.3	5.8±.23	12.39±.48	41.23
7	Eth.ex-400	6	4.31±.17	2.1±.13	5.2±.7	3.4±.39	18.2±.69	60.76
8	Eth.ex-600	6	3.57±.30	1.86±.26	4.71±.36	3.14±.18	20.43±.57	68.09
9	Eth.ex-800	6	2.86±.34	1.43±.20	3.86±.29	2.57±.20	22.42±.48	74.73

Statistical Analysis: The experimental results were expressed as Mean ± SEM data were assessed by the method of analysis of ANOVA followed by student t- test  $p < 0.05$  were considered as statistically significant.

**DISCUSSION:** In the present study, aqueous and ethanolic extracts of *Semecarpus anacardium* fruit at the dose of 300,400,600,800mg/kg. b. wt., tested for antioviulatory activity by studying the estrous cycle. Acute toxicity study was carried out to determine the dose and risk associated with its long term administration. The extract was found to be devoid of acute toxicity upto dose of 2000mg/kg in rats. The reports of the phytochemical studies from other studies have showed the presence of alkaloids hence the antioviulatory activity may be due to the presence active alkaloid principle<sup>14, 15</sup>. But so far not a single plant product is marketed, which can be used as anti-fertility agent in this direction the efforts have been made to study on anti-ovulatory activity of *Semecarpus anacardium* fruit.

Estrogenic activity is shared by many steroidal and non-steroidal compounds. The 3 principal native forms of known endogenous estrogens are, 17-estradiol, estrone and estriol. The most potent biologic form is 17-estradiol, which is used as a component of ocp's for inhibiting gonadotrophin secretion. One of the 1<sup>st</sup> non-steroidal estrogens is diethylstilbestrol, which is structurally similar to estradiol<sup>16</sup>. The non-steroidal compounds with estrogenic activity including flavonoids (flavones, flavonones and isoflavonoids), alkaloids, phenolics occur in a variety of plants are well documented as anti-fertility agents (Anderson LL et al., 1972; Heeshma Khshadani et al, 2006).

It has been observed that, aqueous and ethanolic extracts of *Semecarpus anacardium* fruit in a dose dependent manner 600 and 800 mg/kg. b. wt induces vaginal opening and cornification of vaginal epithelial cells in dose dependent manner .

Many morphological, histological, physiological and biochemical changes occur in the ovary during the estrous cycle. During the maturation of preovulatory follicles, ovulation takes place under the combined and balanced influence of ovarian and extra ovarian hormones. Imbalance in these hormones leads to irregularity in the ovarian functions and duration of estrous cycle<sup>17, 18</sup>. The estrous cycle in the rats treated with 600 & 800 mg/kg. b. wt., showed a decrease in the duration of oestrous and the metoestrous phases. It was also characterised by prolongation of the proestrous

phase/diestrus phase. The prolongation of diestrus phase indicates that maturation of the follicle in the preovulatory phase was delayed, leading to non-maturation of graffian follicle. Non-availability of matured graffian follicle was indicated by reduction in the metaestrous phases. Therefore ovulation was inhibited.

**CONCLUSION:** The results of the present study conclude that the aqueous and ethanolic extracts of *Semecarpus anacardium* fruits have significant reversible antioviulatory activity. The extracts of this plant can be further evaluated for the development of an effective contraceptive agent.

**CONFLICT OF INTEREST:** There are no financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this paper.

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#### DECLARATIONS:

- a) Funding; BRIMS, Bidar
- b) Conflict of interest: none
- c) Ethical approval: taken by the institutional animal ethical committee , BRIMS, Bidar

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