

**ANTIFERTILITY EFFECT OF AERIAL PART OF
CROTALARIA VERRUCOSA IN FEMALE ALBINO
RATS**

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Summary

To evaluate anti-fertility activity of aerial part of *Crotalaria verrucosa* by using 70% ethanolic, 95% ethanolic, and aqueous extracts. The different experimental models used for the evaluation of anti-fertility activity are ;

Estrogenic activity in immature female rats was carried out by taking ethinyl estradiol as standard. Utrine weight, vaginal cornifaction and uterotropic potency determined and biochemical changes in the uterous was determined and compared with control and standard.

Anti-implantation and early abortifacient activity was performed on female rats, the number of implants and resorbtions were compared with control. The 95% ethanolic, 70% ethanolic and aqueous extracts of aerial part of *Crotalaria verrucosa* were found to possess significant estrogenic activity at the dose of 500 mg / kg and 250 mg / kg b.w. as indicated by significant increase in uterine weight, vaginal cornification and utertropic potency like; diameter of the uterus, thickness of the endometrium and height of the endometrial epethelium, and there was also a increase in glucose, cholesterol and alkaline phosphatase in compared to control, but not significantly greater than standard.

The biochemical changes like con. of glucose, cholesterol and alkaline phosphatase is also increase in treated group with plant extract when compare with control group but significantly less than standard group.

Whereas in case of anti-implantation and early abortifacient activity. The 70% ethanolic, aqueous and 95% ethanolic extracts were found to be highly significant when compared to control in dose dependent manner as evident by decrease in number of implants and increase in number of resorbtion. 70% ethanolic and aqueous extracts of *Crotalaria verrucosa* were found to possess highly significant anti-fertility activity in dose dependant manner whereas 95% ethanolic extract was found to be less significant when compared to other two extracts in dose dependent manner.

Key Words: aerial part of *Crotalaria verrucosa*, cholesterol, glucose, alkaline phosphatase.

Introduction

The rapid growth of the world's population over the past one hundred years results from a difference between the rate of birth and the rate of death. The human population will increase by 1 billion people in the next decade. This is like adding the whole population of China to the world's population. The growth in human population around the world affects all people through its impact on the economy and environ-ment. The current rate of population growth is now a significant burden to human well-being. Understanding the factors which affect population growth patterns can help us plan for the future.

It took the entire history of humankind for the population to reach 1 billion around 1810. Just 120 years later, this doubled to 2 billion people (1930); then 4 billion in 1975 (45 years). The number of people in the world has risen from 4.4 billion people in 1980 to 5.8 billion today. And it is estimated that the population could double again to nearly 11 billion in less than 40 years. This means that more people are now being added each day than at any other time in human history [1].

Looking ahead, world population is projected to exceed 6 billion before the year 2000. And according to a report by the United Nation Population fund, total popu-lation is likely to reach 10 billion by 2025 and grow to 14 billion by the end of the next century unless birth control use increases dramatically around the world within the next two decades.

Both death rates and birth rates have fallen, but death rates have fallen faster than birth rates. There are about 3 births for each death with 1.6 births for each death in more developed countries (MDCs)

and 3.3 births for each death in less developed countries (LDCs). The world's population continues to grow by 1 billion people every dozen years.

Rich and poor countries alike are affected by population growth, though the population of industrial countries are growing more slowly than those of developing one. At the present growth rates, the population of economically developed countries would double in 120 years. The Third World, with over three quarters of the world's people, would double its numbers in about 33 years. This rapid doubling time reflects the fact that 37 percent of the developing world's population is under the age of 15 and entering their most productive childbearing years. In the Third World countries (excluding China), 40 percent of the people are under 15; in some African countries, nearly half are in this age group.

The world's current and projected population growth calls for an increase in efforts to meet the needs for food, water, health care, technology and education. In the poorest countries, massive efforts are needed to keep social and economic conditions from deteriorating further; any real advances in well-being and the quality of life are negated by further population growth. Many countries lack adequate supplies of basic materials needed to support their current population. Rapid population growth can affect both the overall quality of life and the degree of human suffering on Earth. Plants are the most important source of medicine. Their application as medicine dates back to prehistoric period. Considerable number of drugs used in modern medicine has figured in ancient manuscripts such as the Rigveda, the Bible, and the Quran. The Ayurveda is a part of Atharvaveda one among the four Vedas. Vedas have revealed that the herbs, shrubs and trees have got life much before the modern life science said it. The ayurveda has laid a scientific foundation for such thinking. Our ancient Philosophers and Biologists like Sushruta, Charaka, and Vagbhata have made investigations on medicinal plants and enriched Ayurveda.

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 plant preparations are reported to have contraceptive properties in ancient Indian literature. But so far no single plant is available which can be developed further as a potent anti-fertility agent. Hence, the search needs to be continued to find out the potent compound [2].

One approach being pursued in the present study is to identify new fertility regulating agents in the search for their presence in natural sources.

Aerial part of *crotalaria verrucosa* reported to contain phytochemical constituents such as, steroids (β -sitosterol), flavonoids, alkaloids, carbohydrates, saponins, tannins and others. Keeping this in view after extensive literature survey available from all scientific sources revealed no information about the pharmacological validation of the anti-fertility activity of Aerial part of *crotalaria verrucosa*. Thus the present study deals with the screening of anti-fertility efficacy by using different experimental model[3,4,5,6,].

Methodology

Plant Material :

Aerial part of *crotalaria verrucosa* was collected in the month of December and February from the fields of Harapanahalli. The authentication was done by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A voucher specimen has been deposited at the museum of our college.

Preparation of Extracts :

Aerial parts of *crotalaria verrucosa* were collected and shade dried. The dried aerial parts were coarse powdered and the powder was packed in to soxhlet column and extracted successively with petroleum ether (60-80°C), 95% ethanol (64.5-65.5°C), 70% ethanol (75°C) and distilled water. The extracts were concentrated by using rotary flash evaporator under reduced pressure. The dried extracts were stored in airtight container in refrigerator below 10°C. The solution of 95% ethanolic, 70% ethanolic and aqueous extracts were prepared using distilled water.

I. Preliminary phytochemical screening :

Based on results of the preliminary phytochemical screening, 70% ethanolic, 95% ethanolic and aqueous extracts have been selected for the following studies.

II. Determination of acute toxicity (LD₅₀).**III. Anti-fertility activity:****1. Estrogenic activity.****2. Anti-implantation and early abortifacient activity.****I). Preliminary Phytochemical Screening.**

The preliminary phytochemical screening was carried out on petroleum ether, 70% ethanol, 95% ethanol and aqueous extracts of aerial part of *crotalaria verrucosa* for the detection of various phytochemical tests for common phytochemical were carried out by standard methods described in practical pharmacognosy by C.K Kokate and K.R. Khandelwal [7].

Animals used :

Female and male albino rats (wistar strain) weighing 150-200gms, immature female rats of 21-23 days old (wistar strain) weighing 40-60gms and albino mice weighing 20-25gms of either sex were used in this study. They were procured from Venkateshwara Animal Suppliers, Bangalore. The animals were acclimatized for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C, relative humidity 65 ± 10% under 12 hours light / dark cycle. The animals were fed with rodent pellet diet (Gold Mohur Lipton India Ltd.) and water ad libitum. Animal ethical clearance for performing the experiments on animals was obtained from the Institutional Animal Ethical Committee (IAEC).

II) Determination of acute toxicity (LD₅₀) The acute toxicity for 95% ethanol, 70% ethanol and aqueous extracts of aerial part of *C.verrucosa* were determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment, fixed dose method was adopted as per OECD Guideline No. 420; (Annexure-2d) of CPCSEA [8].

III) Evaluation of Anti-fertility Activity.

1) Estrogenic activity on immature female rats [9]

Immature female rats of wistar strain 21-23 days old weighing 40-60 gms were used. They were divided in to eight groups of six animals each. The various groups were treated as follows:

Group I - Control: (Vehicle Tween 80, 1%, 5 ml/kg.) p.o.

Group II - Standard: Ethinylestradiol 1 µg/rat/day in olive oil i.m.

Group III - 95% ethanolic extract of aerial part of *c. verrucosa* 250mg/kg p. o.

Group IV - 95% ethanolic extract of aerial part of *c. verrucosa*
500mg/kg p. o.

Group V - 70% ethanolic extract of aerial part of *c. verrucosa*
250mg/kg p. o.

Group VI - 70% ethanolic extract of aerial part of *c. verrucosa*
500mg/kg p. o.

Group VII - Aqueous extract of aerial part of *c. verrucosa*
250mg/kg p.o.

Group VIII - Aqueous extract of aerial part of *c. verrucosa*
500mg/kg p.o.

All the above treatments were given for 7 days. Vagina and the vaginal smears were examined in all the animals in the treated groups for 7 days of treatment. 24 hrs. of last treatment all the animals were sacrificed by decapitation and uteri were dissected out, cleared off the adhesive tissue, blotted on filter paper and weighted quickly on a sensitive balance. The tissues were fixed in Bouin's fixative for 24 hrs. Dehydrated in alcohol and embedded in paraffin. The paraffin blocks were sectioned at 6 μ and stained with haemotoxylene-eosin solution (H & E Stain) for histological observations. The diameter of the uterus, thickness of endometrium, and the height of endometrial epithelium were measured in 10 randomly selected sections using a calibrated ocular micrometer.

a) Biochemical changes in the uterus

The other portion of the uterus was homogenized with ice cold distilled water in a pre-cooled mortar and pestle to contain 10 mg of tissue per ml. the homogenate was centrifuge in cold at 3000 r.p.m for 15 minutes and the supernatant was used for the estimation of glucose, cholesterol and alkaline phosphatase using in the standard methods (Varley et al., 1984)

Statistical analysis :

Results were expressed as mean \pm SEM, (n=6). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test by using Graph Pad In stat software. P value less than 0.05 was considered to be statistically significant. *P<0.05, **<0.01 and ***,0.001, when compared with control and toxicant group as applicable.

2) Anti-implantation and early abortifacient activity in rats.

The method was adopted with the modification for the anti-implantation and early abortifacient activities of 95% ethanol, 70% ethanol and aqueous extracts of aerial part *c. verrucosa* Female

albino rats (Wistar strain) weighing 150-200gms were used to assess anti-implantation and early abortifacient activity. All the animals were maintained under controlled standard animal house condition with access to food and water ad libitum. A vaginal smear from each rat was monitored daily. Only the rats with normal oestrous cycles were selected for the experiment.

Female rats of proestrus phase were kept with male rats of proven fertility for mating in a ratio of 2:1. The females were examined, the following morning for evidence of copulation. The animals exhibiting thick clumps of spermatozoa in vaginal smears were separated from male partner. That day when spermatozoa were detected in the vaginal smear was considered as day one of gestation[10,11,12].

The separated pregnant rats were divided into seven groups of six rats each.

The various groups were treated as follows:

- Group I - Control: (Vehicle Tween 80, 1%, 5 ml/kg.) p.o.
- Group II - 95% ethanolic extract of aerial part of *c. verrucosa* 250mg/kg p. o.
- Group III - 95% ethanolic extract of aerial part of *c. verrucosa* 500mg/kg p. o.
- Group IV - 70% ethanolic extract of aerial part of *c. verrucosa* 250mg/kg p. o.
- Group V - 70% ethanolic extract of aerial part of *c. verrucosa* 500mg/kg p. o.
- Group VI - Aqueous extract of aerial part of *c. verrucosa* 250mg/kg p.o.
- Group VII - Aqueous extract of aerial part of *c. verrucosa* 500mg/kg p.o.

The extracts were administered orally from first day to seventh day of gestation. The control animals received only vehicle. On the tenth day laprotomy was carried out under light ether anesthesia in semi sterile condition. The uteri were examined to determine the number of implantation sites. The numbers of corpora lutea in ovaries were also recorded. The abdomen was sutured and animals left in cages. The drugs were administered orally again for 3 days (day 14 to 16). On the eighteenth day laprotomy was carried out once again under light ether anesthesia for the abortifacient study.

The percentages of anti-implantation and early abortifacient activities were calculated by using following formula.

$$\% \text{ of anti-implantation activity} = 100 - \left[\frac{\text{No. of implantation}}{\text{No. of Corpora lutea}} \times 100 \right]$$

$$\% \text{ of abortifacient activity} = \frac{\text{No. of resorptions}}{\text{No. of Corpora lutea}} \times 100$$

Preparation of extract and properties:

Successive soxhlet extract process yielded 1.004% yellow waxy colored pet. ether extract, 13.38% of dark brown colored 95% ethanolic extract, 15.03% of dark brown colored aqueous extract. In addition to that hydro alcoholic extract (30:70) was also prepared. Hydro-alcoholic extract was dark brown in color and the yield was 18.14%.

Determination of acute toxicity (LD₅₀)

The acute toxicity studies of 95% ethanolic, 70% ethanolic (Hydro-alcoholic) and aqueous extracts of aerial part of *c. verrucosa* were found to be safe and no mortality was found at doses of 2000 mg/kg b.w. Hence 2500 mg/kg was LD₅₀ cutoff value for all the above extracts.

So, that the doses selected for all the extracts as per OECD guideline No. 420 (Annexure 2d) fixed dose method.

Evaluation of anti-fertility activity:

1) Estrogenic activity on immature female rats :

a) Gravimetric changes :

The effect of 95% ethanolic, 70% ethanolic and aqueous extract on immature female rat uterus is shown in Table No. 1.

Oral administration of the test extracts caused a significant increase in uterine weight in dose dependent manner, when compared to those of control rats.

The 70% ethanolic extracts at dose of 500 mg/kg b.w., aqueous extracts at dose of 500 mg/kg b.w. and 95% ethanolic extracts at the dose of 500 mg/kg b.w. were found to possess highly significant (P<0.001) estrogenic activity as indicated by increase in the uterine weight of immature female rats when compared to control, when

compared to standard found to possess lesser effect than that of standard.

The 70% ethanolic extract at dose of 250 mg/kg b.w., aqueous extract at dose of 250 mg/kg b.w. and 95% ethanolic extract at dose of 250mg/kg b.w. were found to possess significant ($P<0.01$) estrogenic activity as indicated by increase in the uterine weight of immature female rats, when compared to control.

b) Vaginal changes :

Oral administration of all the test extract at the dose of 500 mg/kg b.w. showed vaginal opening and the smear showed proestrous or estrous conditions.

The number of cornified cells in vaginal smear was considerably higher (+ to ++) than that of control (0 to +) but notably less than that of standard (+++).

c) Micrometric changes in the uterus :

All the test extracts at the dose of 250 mg/kg b.w. shows significant uterotrophic responses are shown in table including diameter of the rat uterus ($P<0.01$) thickness of the endometrium ($P<0.01$) and height of the endometrial epithelium ($P<0.01$) when compared to those of control animals.

All the test extracts at the doses of 500 mg/kg b.w. shows that highly significant uterotrophic responses including. Diameter of the rat uterus ($P<0,001$) thiciness of the endometrium ($P<0.001$) and height of endometrial ephelium ($P<0.001$) when compared to those of control rats.

The above results obtained with test extracts on uterine weight and uterotrophic responses in immature female rats also supported by histological architecture shown in table no. 2.

d) Biochemical changes.

All the test extracts at the dose of 250 mg/kg b.w. shows significant Biochemical changes in the uterus are shown in table including con. Of glucose, alkaline phosphatase, and cholesterol when compared to control animals but significantly less than the standard treated group.

All the test extracts at the doses of 500 mg/kg b.w. shows that highly significant Biochemical changes in the uterus are shown in table including con. Of glucose, alkaline phosphatase, and cholesterol when compared to control animals but significantly less than the standard treated group fig No. 1-8.

Statistical analysis was carried out using analysis of variance (ANOVA) test. The results were judged significant if ($P<0.05$).

2) Anti-implantation and early abortifacient activity

A dose dependent anti-implantation and early abortifacient activity of the 95% ethanolic, 70% ethanolic and aqueous extracts was evident by significant decrease in number of implantation sites and increase in number of resorptions, when compared with the control group.

The percentage of anti-fertility activity of the 95% ethanolic extract at dose of 250 mg/kg b.w. and 500 mg/kg b.w. were found to be 42.6% and 48.0% respectively in dose dependent manner, when compared to control, where as percentage of anti-fertility activity of 70% ethanolic extract at dose of 250 mg/kg b.w. and 500 mg/kg b.w. were found to be 57.2% and 65.14% respectively, when compared to control.

However, the percentage of anti-fertility activity of aqueous extract at dose of 250mg/kg b.w and 500mg/kg b.w. were found to be 52.9% and 56.4% respectively, when compared to control the results are shown in the Table No.3

Results

Table No. 1

Estrogenic activity of various extracts of aerial part of *Crotalaria verrucosain* immature female rats

Sl. No.	Treatment Extracts / Drugs	Dose mg/kg	Uterine wt. in mg Mean \pm SEM	Vaginal Status	Vaginal cornification
1	Control (Vehicle)	5 ml/kg	43.62 \pm 2.202	Not opened	Nil
2	Ethinyl estradiol	1 μ g/ rat/day	156.2 \pm 1.498***	Opened	+++
3	70% ethanolic extract	500	108.2 \pm 3.494***	Opened	+++
4	70% ethanolic extract	250	74.32 \pm 1.825**	Opened	+++
5	Aqueous Extract	500	85.89 \pm 1.379***	Opened	+++
6	Aqueous Extract	250	62.38 \pm 2.185**	Not Opened	++
7	95% ethanolic extract	500	73.42 \pm 0.8820**	Opened	+++
8	95% ethanolic extract	250	57.88 \pm 0.9315**	Not Opened	++

Values are the Mean \pm S.E.M. of six rats / treatment
Significance **P<0.01 (n=6) ***P<0.001 (vs. Control).

+ = Nucleated epithelial cells

++ = Nucleated epithelial cells & cornified cells

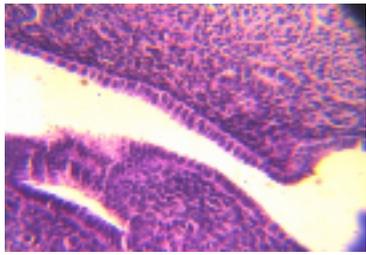
+++ = Cornified cells

Table No. 2

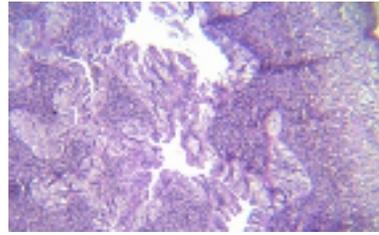
Micrometric changes in the uterus due to administration of various extracts of aerial part of *Crotalaria verrucosa*

Sl. No.	Treatment Extracts / Drugs	Dose mg/kg	Diameter of Uterus (μm)	Thickness of Endometrium (μm)	Epithelial cell height (μm)
1	Control (Vehicle)	Tween 80 (1%, 5 ml/kg)	641.8 \pm 2.190	341.0 \pm 1.498	10.00 \pm 0.258
2	Ethinyl estradiol (Estradiol valerate)	1 $\mu\text{g}/\text{rat}/\text{day}$	1820.0 \pm 6.802***	641.4 \pm 2.255***	17.83 \pm 0.2789***
3	70% ethanolic extract	500 mg	1114.0 \pm 23.56***	576.6 \pm 2.318***	15.00 \pm 0.2887***
4	70% ethanolic extract	250 mg	825.1 \pm 4.369**	400.3 \pm 3.630**	12.50 \pm 0.428**
5	Aqueous Extract	500 mg	890.6 \pm 2.939***	431.3 \pm 1.521***	13.00 \pm 0.2887***
6	Aqueous Extract	250 mg	851.5 \pm 2.876**	276.9 \pm 4.32**	11.50 \pm 0.4655 ^{ns}
7	95% ethanolic extract	500 mg	801.81 \pm 2.281**	405.6 \pm 2.256***	12.42 \pm 0.273***
8	95% ethanolic extract	250 mg	748.1 \pm 2.972**	356.5 \pm 1.419**	11.33 \pm 0.557 ^{ns}

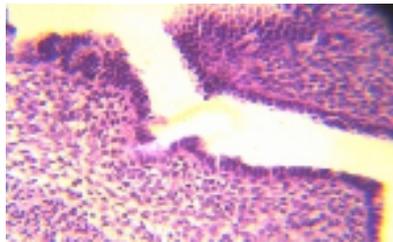
Significance **P<0.01 (n=6) ***P<0.001 (vs. Control).
ns = Not Significance



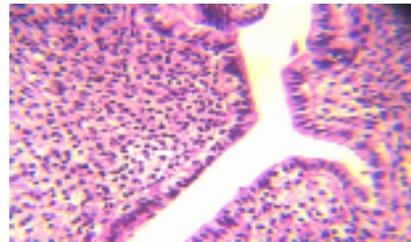
Control group



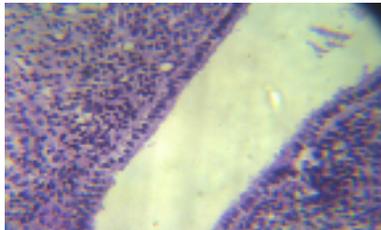
standard group



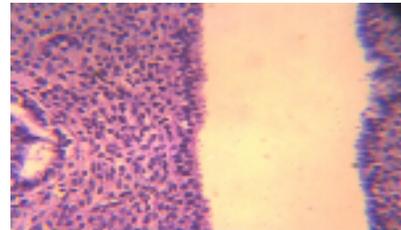
70% ethanolic extract
500mg/kg



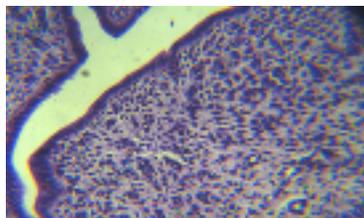
70% ethanolic extract
250 mg/kg



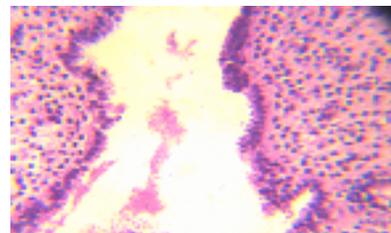
aqueous extract
500 mg/kg



aqueous extract
250mg/kg



95% ethanolic extract
500 mg/kg



95% ethanolic extract
250mg/kg

Table No. 3

Biochemical changes in the uterus due to administration of various extracts of aerial part of *Crotalaria verrucosa*

Sl. 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)	0.5033 ± 0.02906	0.3217 ± 0.006009	4.342 ± 0.03177
2	Ethinyl estradiol (Estradiol valerate)	1 µg/rat/day	1.302 ± 0.02892***	0.8100 ± 0.006009***	7.053 ± 0.05760***
3	70% ethanolic extract	500 mg	1.092 ± 0.03005***	0.7012 ± 0.00503***	6.012 ± 0.03280***
4	70% ethanolic extract	250 mg	0.8867 ± 0.04944**	0.6467 ± 0.01054**	5.335 ± 0.05661**
5	Aqueous Extract	500 mg	1.222 ± 0.1358***	0.5983 ± 0.006009***	5.922 ± 0.03049***
6	Aqueous Extract	250 mg	0.8050 ± 0.02604**	0.5100 ± 0.005773**	5.028 ± 0.01167**
7	95% ethanolic extract	500 mg	0.7000 ± 0.00577***	0.4033 ± 0.01116***	4.915 ± 0.01857***
8	95% ethanolic extract	250 mg	0.5967 ± 0.006667**	0.3850 ± 0.004282**	4.585 ± 0.02320**

Values are the Mean ± S.E.M. of six rats / treatment.

Significance **P<0.01 (n=6) ***P<0.001 (vs. Control).

Table No. 4

effect of various extracts of aerial part of *Crotalaria verrucosa* on anti-implantation and early abortifacient activity

Group and Treatment	Animals Used	Anti-implantation		Early Abortifacient	% of Anti-implantation activity	% of Early Abortifacient activity	% of Anti-fertility activity
		No. of Implantation	No. of Corpora-lutea	No. of Resorbed Implantation			
Control (vehicle)	6	11	13	-	15.3	0.00	15.3
		13	15	-			
		12	14	-			
		14	16	-			
		11	13	-			
		11	14	-			
70% ethanolic extract (500mg/kg)	6	5	12	1	57.9	7.24	65.14
		6	11	-			
		5	13	1			
		4	10	1			
		4	13	2			
		5	10	-			
70% ethanolic extract (250mg/kg)	6	6	13	-	51.4	5.8	57.2
		5	12	1			
		5	10	1			
		6	11	1			
		4	12	-			
		6	10	1			
Aqueous Extract (500mg/kg)	6	5	13	1	52.1	4.34	56.4
		6	11	0			
		7	13	1			
		5	12	-			
		4	10	1			
		6	13	0			
Aqueous Extract (250mg/kg)	6	6	11	-	50.0	2.9	52.9
		7	13	1			
		5	11	-			
		5	10	-			
		6	11	1			
		5	12	-			

95% ethanolic extract (500mg/kg)	6	5 8 7 6 5 7	11 12 13 11 11 10	- 1 1 - 1 -	44.0	4.4	48.0
95% ethanolic extract (250mg/kg)	6	8 7 6 7 6 7	12 13 11 11 10 11	1 - - 1 - -	39.7	2.9	42.6

Discussion

The plant kingdom is a rich source of biologically active agents, revealing various types of pharmacological activities. India has a rich heritage, use of medicinal plants for contraceptive activities in female as well as in male. Since last few decades plants have been systematically screened for a variety of fertility regulating activities like, anti-ovulatory, anti-implantation abortifacient, estrogenic / anti-estrogenic in female and anti-spermatogenic, anti-steroidogenic, androgenic / anti-androgenic and spermicidal activities in males [13].

Research on fertility regulating plants has been given priority by Central Drug Research Institute (CDRI) Lucknow and Indian Council of Medical Research (ICMR) New Delhi, in recent years, 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 on the anti-fertility activity of aerial part of *c. verrucosa*.

The preliminary phytochemical investigation on aerial part of *c. verrucosa* revolves the presence of carbohydrates, flavonoids, steroids, tannins and saponins in 95% ethanolic, 70% ethanolic and aqueous extracts, but these phytochemicals were found to be absent in petroleum ether extract. The percentage yield of the petroleum ether extract was also found to be very less, when compared to 95% ethanolic, 70% ethanolic and aqueous extracts.

Hence, 95% ethanolic, 70% ethanolic and aqueous extracts of aerial part of *c. verrucosa* were taken for evaluation of the anti-fertility activity.

The data obtained in the present study indicates that 70% ethanolic, aqueous and 95% ethanolic extract of aerial part of *c. verrucosa* exhibited more significant anti-fertility activity in dose dependent manner.

95% ethanolic, 70% ethanolic and aqueous extracts at dose of 500 mg/kg b.w., 250 mg/kg b.w. were found to possess highly significant estrogenic activity as indicated by increase in uterine weight, vaginal cornification and uterotrophic responses. In immature female rats, when compared to control, but not significantly greater than standard in dose dependent manner.

In the present study 70% ethanolic and aqueous extracts at the dose of 250 mg/kg b.w. and 500 mg/kg b.w. tested for anti-implantation and abortifacient activity exhibited a significant decrease in number of implantation sites and increase in number of resorptions in a dose dependent manner, whereas 95% ethanolic extract exhibited less significant anti-implantation and abortifacient activity, when compared to other extracts in dose dependent manner.

Estrogenic activity is shared by many steroidal and non-steroidal compounds. The three principal native forms of known endogenous estrogens are 17- β estradiol, estrone and estrinol. The most potent biologic form is 17- β estradiol, which is used as a component of oral contraceptives for inhibiting gonadotropin secretion.

One of the first non-steroidal estrogen is diethylstilbestrol, which is structurally similar to estradiol. The non-steroidal compounds with estrogenic activity including flavonoids (flavones, flavonones and isoflavonoids) alkaloids, phenolics, occur in variety of plants are well documented as anti-fertility agents [14,15].

Reproductive cycle in mammals commences with the onset of puberty and in laboratory animals like rats, it is usually judged with the help of vaginal opening at about 38 days of age. Reproductive and general metabolic effects in mature and immature rats are manipulated with the ingestion of phytoestrogenic substances that produces effects similar to that of gonadal steroid 17 β -estradiol.

It has been observed that 70% ethanolic, aqueous and 95% ethanolic extracts of aerial part of *c. verrucosa* at dose of 250 mg/kg b.w. and 500 mg/kg b.w. provoked significant increase in the uterine weight,

induces vaginal opening and cornification of vaginal epithelial cells and increases the uterotrophic potency in dose dependent manner.

In the present study the histological evidence of the uterus treated with 95% ethanolic, 70% ethanolic and aqueous extracts clearly supports an unfavorable uterine milieu, showing obliterated lumen with loose stroma, increased height of luminal epithelium and stimulated uterine gland in respective extracts, therefore from the present findings it can safely be said that all the extracts possess estrogenic activity in dose dependent manner.

Anti-implantation and early abortifacient activity :

Implantation in rat depends on the completion of basic sequence of events occurring both in fertilized egg and endometrium.

The fertilization takes place in the fallopian tube and then the developing ovum following the above pattern enters the uterus. Different extracts of aerial part of *C. verrucosa* administered orally from day 1 to 7 of pregnancy in rats exhibited highly significant loss of implants suggesting anti-implantation activity. The highest loss of implants was with 70% ethanolic extract at a dose of 500 mg/kg b.w. This effect may be due to the imbalance in the estrogen-progesterone environment. Pituitary hormones are essential for the first 11 days of pregnancy; progesterone, a pregnancy hormone secreted by the corpora lutea, is sustained by reduction with FSH through day 1-7, LH becomes the important luteotropic hormone from day 8-12 of pregnancy to maintain the progesterone secretion of the corpora lutea and thereafter the placenta will take over the function. The level of estrogen secretion during pregnancy is comparatively lower, compared to progesterone as the former is in the range of nanograms and later in micrograms, throughout pregnancy except near term [15,16,17].

Thus progesterone is the main hormone to maintain pregnancy, the synergistic action of estrogen and progesterone during gestation is necessary for the maintenance of pregnancy successfully. In several species including many non-humans, progesterone and estrogen synergistic action is essential for blastocyst implantation and for the maintenance of pregnancy in all phases.

In the present study the anti-implantation and abortifacient activity of the extract is mainly due to its confirmed estrogenic activity; a high dose of estrogen disproportionate to progesterone leads to the resorption of fetuses [18,19].

The foetal loss in the present study is mainly due to the resorption of embryos because of absence of vaginal bleeding. The persistence of placentomas in the uterus observed on 18th day of pregnancy also supports that foetal loss is mainly due to resorption, it is evident from the above facts that the 95% ethanolic, 70% ethanolic and aqueous extracts contains the compounds which are anti-implantation and abortifacient [20,21].

Thus the data obtained from phytochemical and pharmacological evaluations of aerial part of *C. verrucosa* tend to suggest that 70% ethanolic and aqueous extract possess significant and 95% ethanolic extract possess less significant estrogenic, anti-implantation and early abortifacient activity in a dose dependent manner.

Conclusions

The present study of anti-fertility activity was carried out on 70% ethanolic, 95% ethanolic and aqueous extracts of aerial part of *C. verrucosa* by using estrogenic anti-implantation and abortifacient models.

The 70% ethanolic and aqueous extracts of aerial part of *C. verrucosa* were found to possess highly significant and 95% ethanolic extract found to possess less significant anti-fertility activity against estrogenic anti-implantation and abortifacient experimental models in dose dependent manner

The estrogenic activity of 70% ethanolic and aqueous extracts and 95% ethanolic extract at the dose 500 mg/kg b.w. were found to possess significant estrogenic activity as indicated by increase in uterine weight, vaginal cornification and uterotrophic potency in immature female rats when compared to control, but not significantly greater than the standard.

The biochemical changes like con. of glucose, cholesterol and alkaline phosphatase is also increase in treated group with plant extract when compare with control group but significantly less than standard group

The anti-implantation and early abortifacient activity of 70% ethanolic and aqueous extracts of aerial part of *C. verrucosa* at a dose of 500 mg/kg b.w. were found to be more significant when compared with other doses of the extracts. Where as 95% ethanolic extract showed less significant anti-implantation and early abortifacient activity when compared to other two extracts in a dose dependent manner.

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