

## **Antifertility Effect of Aqueous, Ether and Chloroform Extract of *Caesalpinia Pulcherrima* on Female Albino Rats**

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**Abstract:** *The present work deals with antifertility effect of the aqueous, ether, chloroform extract of *Caesalpinia Pulcherrima* (arial part) in female albino rats. Pregnant rats were randomized into 10 groups [A to D]. Rats were laprotomised on 10<sup>th</sup> day of pregnancy and the live fetuses were observed in both the horns of the uterus. Rats in group A (control) were orally administered, once daily with 0.5 ml of distilled water while those in group B to D served as experimental groups and were administered 100, 200 and 400 mg/kg body weight doses of the aqueous, ether, chloroform extract of *Caesalpinia Pulcherrima* (arial part) respectively. It was found that the extract reduced the number of live fetuses, whereas the resorption index and post implantation losses increased significantly. The % abortion was found to be highest (100%) with 400 mg/kg dose of chloroform extract of *Caesalpinia Pulcherrima* (Aerial part).*

**Keywords:** *Post coital antifertility, antifertility effect, *Caesalpinia pulcherrima*, Estrous cycle and Female albino rats.*

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### **1. INTRODUCTION**

The oral contraceptive agent that can control human fertility is as old as recorded history though wide variety of synthetic contraceptive agent are available [1], these can't be used continuously due to their several side effect. [2] Several plants have been confirmed as antifertility, abortive, uterine stimulant, estrogenic or cytotoxic agents in animals and humans [3]. Hence people are now looking back to age old tradition of using herbal medicines which have minimum side effect. *Caesalpinia Pulcherrima* is perennial large shrub or small tree found throughout area of amravati region. It has several medicinal properties used in the treatment of ulcer, fever, tumors, asthma and skin diseases [4]. *C. pulcherrima* is also used in folk medicines the stem is used as an abortifacient and emmenagogue, while decoctions of the bark, roots and bark are used as a febrifuge and to treat liver disorders as well as ulcers from mouth and throat [5]. Previous studies on this plant have resulted in the isolation of several diterpenoids [6-7], flavonoids [8]. flavonoids [9], homoisoflav [10], homoisoflavonoids [11]. Some of the constituents were found to possess antitumour [12], antimicrobial properties [13].

But to the best of our knowledge, there is no information in the open scientific literature that has substantiated or refuted the antifertility claims *Caesalpinia Pulcherrima* (aerial part) in the folklore medicine. Therefore plant present work was undertaken to validate scientifically the antifertility role of *Caesalpinia pulcherrima* (Aerial part) as acclaimed by the traditional tribal user in melghat area.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Plant Material

The plant *Caesalpinia Pulcherrima* was collected from surrounding region of Vidarbha Institute of Science and Humanities, Amravati (M.S), and identified and authenticated by experts from Botanical Survey of India, Pune. A voucher specimen of the plant has been deposited in their herbarium. (VADCAP2)

### 2.2. Preparation of Extract

The arial part of *Caesalpinia Pulcherrima* were collected, shade dried, powdered and subjected to soxhlet extraction successively with distil water, ether (40-60<sup>0</sup>) and chloroform. The extract was evaporated to near dryness on a water bath, weighed and kept at 4<sup>0</sup> c in refrigerator until the experimental testing.

### 2.3. Phytochemical Screening

The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as described by Thimmaiah[14].

### 2.4. Procurement and Rearing of Experimental Animal

Albino rats (Wistar strain) used in the present investigation was procured from S.N. Institute of Pharmacy, Pusad (M.S). The rats were acclimatized for 15 days to the best laboratory condition (prior to experiment) and maintained on balanced diet (Trimurti lab feeds, Nagpur). Water was provided *ad libitum*.

### 2.5. Acute Toxicity Study

The animals were divided into three groups and the extract was administered orally at the doses of 500, 1000 and 2000 mg/kg body weight separately. Control rats received the vehicle only. The rats were observed for 72 hr. for behavioral changes and mortality[15].

### 2.6. Antifertility Testing

The plant extract were tested in female albino rats for abortifacient activity by the method as described by Khanna and Chaudhary[16]. The vaginal smears of caged female rats of known fertility were monitored daily. Unstained material was observed under a light microscope. The female rats were caged with males of proven fertility in the ratio of 2:1, in the evening and examined the following day for the evidence of copulation. Rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as 1<sup>st</sup> day of pregnancy. These rats were randomly distributed into 4 groups, a control group and three experimental groups of 6 animals each. On the 10<sup>th</sup> day of pregnancy animals were laprotomised under light ether anesthesia using sterile condition. The two horns of uteri were examined to determine the implantation sites. There after the abdominal wound was sutured in layers. Post operational care was taken to avoid any infection.

The extract to be tested were then fed to operated pregnant rats i.e. extract of *Caesalpinia Pulcherrima* at doses 100 mg/kg, 200 mg/kg, 400 mg/kg of each extract specified, by an intragastric (i.g.) soft rubber catheter from day 11 up to the 15<sup>th</sup> day. The animals were allowed to go full term. After delivery the pups were counted and the antifertility activity of extract was evaluated. Litters were examined for any malformations.

### 2.7. Effect on Estrous Cycle

The aqueous, ether and chloroform extract at dose of 400 mg/kg body weight was found to be active amongst the three treatments in antifertility testing .Hence it was subjected to a detailed investigation for study of estrous cycle. The studies were conducted on adult female rats for 30 days. To study the estrous cycle pattern, animal showing regularity in the normal cycle were separated and chosen for further studies. Those animals showing normal estrus cycle were divided in two groups of 6 animals each; Group I- (control), received distilled water (Vehicle) and Group – (treated), received aqueous, ether, chloroform extract at dose of 400 mg/kg body weight. Vaginal smear using saline solution were taken twice daily during the entire treatment period, observation of the vaginal opening and the cell type obtained in a vaginal smear was also done.

## Antifertility Effect of Aqueous, Ether and Chloroform Extract of *Caesalpinia Pulcherrima* on Female Albino Rats

The duration of estrous cycle together with that of various phases was determined [17, 18] the proportion among the cells observed was used for determination of the estrous cycle phases [19].

All procedures with animals were conducted strictly in accordance with approved guideline regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, Ministry of Justice and Empowerment, Government of India. During the experiments, maximum care was taken to minimize animal suffering. All experimental protocols were met with the approval of Institutional Animal Ethics Committee registration number. (SGBAU/Ph. D/Zoo/10/2009, w.e.f. 15.07.2009)

### 2.8. Effect on Body Weight and Reproductive Organ Weight

After 30 days treatment of aqueous, ether and chloroform extract at dose of 400 mg/kg body weight, all the control and experimental groups of female rats were evaluated for any changes in their body weight as well as for their reproductive organ weight by the method of Amini and Kamkar[20].

### 2.9. Statistical Analysis

All the data are expressed as mean $\pm$ SEM. Statistical analysis was done by using paired and unpaired student's t-test[21].

## 3. RESULTS

### 3.1. Phytochemical Scening

Preliminary phytochemical screening of the extract aqueous, ether, chloroform *Caesalpinia Pulcherrima* revealed the presence of alkaloids, flavonoids, steroids, tannins and saponines where as anthraquinone were not detected (Table-1).

**Table 1.** Phytochemical profile of *Caesalpinia Pulcherrima* ( arial part) extract

Name of the plant	Alkaloids	Anthraquinone	Flavonoids	SimplePhenolics	Steroids	Tannins	Saponins
<i>Caesalpinia Pulcherrima</i> ( arial part)	+	-	+	+	+	+	+

+ Present, - Absent

### 3.2. Acute Toxicity Study

Toxicity symptoms such as respiratory distress, salivation, weight loss and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment. Similarly no mortality and changes in the behavior were observed in all treated and control groups of the rats up to a dose of 1000, 2000, 4000 mg/kg body weight. Hence one-tenth of this dose was used for antifertility testing.

### 3.3. Antifertility Study

The aqueous, ether, chloroform extract of *Caesalpinia Pulcherrima* ( arial part) when evaluated for their antifertility activity, were found to exhibit significant pregnancy interceptive activity. Administration of 100mg/kg, 200 mg/kg 400 mg/kg body weight of the aqueous, extract resulted in respectively shows 17.64%, 34.69 %, 64.69% abortifacient activity respectively (Table-2) and 100 mg/kg, 200 mg/kg, 400mg/kg body weight of the ether extract resulted in 25 %, 32.72%, and 49.15% % abortion respectively (Table-3). And chloroform extract of 100mg/kg, 200 mg/kg, and 400mg/kg body weight shows 51.61%, 69.64 %, 100% abortion respectively (Table-4) this was evident from decreases in the percentage of live fetuses. While no live fetus was observed in 400 mg/kg body weight of chloroform extract. The percent resorption index increased from zero in the control to 64.69%, 49.15% and 100% in the 400mg/kg body weight of aqueous, ether, chloroform extract treated animals respectively.

**Table 2.** Effect of aqueous of *Caesalpinia Pulcherrima* (arial part) on fertility of female rats when fed orally from day 11 to 15 of pregnancy

Treatment Groups	Body Weight (gm)	Drug Dose (mg/kg of body wt)	Sample Size	No. of fetus observed in individual rats on day 10	No. of fetus delivered (Litter Size)	No. of resorption in individual rats	No. of resorption in Mean±S.E	% abortifacient activity
Group- A Control/ (Vehicle)	140-200	-----	6	6(10,9,8,8,9,1)	6(10,9,8,8,9,1)	0,0,0,0,0,0	0	Nil
Group- B Aqueous extract	170-210	100	6	6(9,10,8,7,8,9)	6(7,8,8,6,6,7)	2,2,0,1,2,2	1.5±0.83**	17.64. %
Group-c Aqueous extract	170-210	200	6	6(8,7,10,7,9,8)	6(6,4,6,5,7,4)	2,3,4,2,2,4	2.83±0.39**	34.69 %
Group- D Aqueous extract	170-210	400	6	6(11,7,8,11,9,10)	6(5,4,3,7,5,6)	6,3,5,4,4,4	4.33±0.42***	46.42%

Values in Mean±S.E. (Standard error), n=6, \*P<0.05, \*\*P<0.01, \*\*\*P<0.00400mg/kg

**Table 3.** Effect of ether extract of *Caesalpinia Pulcherrima* (arial part) on fertility of female rats when fed orally from day 11 to 15 of pregnancy

Treatment Groups	Body Weight (gm)	Drug Dose (mg/kg of body wt)	Sample Size	No. of foetus observed in individual rats on day 10	No. of rats delivered (Litter Size)	No. of resorption in individual rats	No. of resorption in Mean±S.E	% abortifacient activity
Group- A Control (Vehicle)	140-200	-----	6	6(10,9,8,8,9,12)	6(10,9,8,8,9,12)	0,0,0,0,0,0	0	Nil
Group- B Ether extract	160-180	100	6	6(11,9,8,8,10,9)	6(9,6,7,8,6,6)	2,2,2,1,4,3	2.33±0.42***	25. %
Group-c Extract	160-180	200	6	6(12,10,9,7,9,8)	6(9,6,6,5,5,6)	3,4,3,2,4,2	3±0.36*	32.72 %
Group- D Aqueous extract	160-180	400	6	6(9,11,12,10,9,8)	6(5,7,6,4,4,3)	4,4,6,6,5,5	5±0.36***	49.15%

Values in Mean±S.E. (Standard error), n=6, \*P<0.05, \*\*P<0.01, \*\*\*P<0.00

**Table 4.** Effect of chloroform extract of *Caesalpinia Pulcherrima* (arial part). On fertility of female rats when fed orally from day 11 to 15 of pregnancy

Treatment Groups	Body Weight (gm)	Drug Dose (mg/kg of body wt)	Sample Size	No. of fetus observed in individual rats on day 10	No. of fetus delivered (Litter Size)	No. of resorption in individual rats	No. of resorption in Mean±S.E	% abortifacient activity
Group- A Control/ (Vehicle)	140-200	-----	6	6(10,9,8,8,9,12)	6(10,9,8,8,9,12)	0,0,0,0,0,0	0	Nil
Group- B Chloroform extract	150-170	100	6	6(10,12,12,7,10,11)	6(4,6,7,3,5,5)	6,6,5,4,5,5	5.33±0.33***	51.61%
Group-c Chloroform extract	150-170	200	6	6(9,10,12,8,9,8)	6(4,3,5,3,4,2)	5,7,7,5,5,6	6.5±0.22***	69.64 %
Group- D Chloroform extract	150-170	400	6	6(7,9,8,7,10,9)	6(0,0,0,0,0,0)	7,9,8,7,10,9	8.66±0.42***	100%

Values in Mean±S.E. (Standard error), n=6, \*P<0.05, \*\*P<0.01, \*\*\*P<0.00

### 3.4. Effect on Estrous Cycle

In the present study, aqueous, ether, and chloroform extract of *Caesalpinia Pulcherrima* at 400 mg/kg body weight shows prolongation of estrous cycle and diestrus phase particularly in

experimental animals. The prepared smear was examined microscopically under low power for different types of cells. Four phases of the estrous cycle were identified depending upon the presence of cell types found in the smear. If majority of cells were leukocytes, then it was labeled as diestrus phase. Presence of large number of nucleated cells indicated proestrus phase. Estrus phase was confirmed when the smear showed more than 50% cornified epithelial cells. Metestrus phase was indicated by the presence of many neutrophils and scattered squamous epithelial cells in the smear

### **3.5. The Effect of Body Weight and Reproductive Organ Weight**

The administration of the 400 mg/kg body weight of aqueous, ether, chloroform extract of *Caesalpinia Pulcherrima* (arial parts) did not significantly change the body weight of the experimental animals. but there was a significant decrease in weight of ovary and increase in uterine weight in treated rats as compared with control . These result may suggest that high dose of aqueous, ether, chloroform extract of *Caesalpinia Pulcherrima* (arial parts) possesses estrogenic activity which might be responsible for fetus resorption. .

## **4. DISCUSSION**

Preliminary phytochemical studies indicated the presence oftannins, flavonoids, triterpenoids andapthaquinone in the acetone extract. According to the literatures flavonoids and plumbagin (naphthaquinone) are known to exhibit anti fertility activity [22, 23, 24] in our study also the abortifacient activity of *Caesalpinia Pulcherrima* also may be due to the presence of flavonoids. Previous reports indicate the presence of flavonoids [25] and terpenoids in medicinal plants with contraceptive or pregnancy interceptory effects. Preliminary phytochemical studies of the *H. acida* stem bark extract indicated the presence of a tannin, flavonoids saponins, steroids which are reported to have contraceptive activity [26, 27]. Aqueous extracts of the roots of both plants *Moringa oleifera*. and *Moringa coneansensis* and of the bark of *M. oleifera* are effective in preventing implantation in rats [28].

Daily administration of aqueous, petroleum ether, and chloroform extracts of *Caesalpinia Pulcherrima* 100 ,200 400 mg/kg body weight produce a dose dependent adverse effect on pregnancy showing significant abortifacient activity. These results are in agreement with those of Petroleum ether and dichloromethane leaf extracts of *Inula viscosa* which exhibited pronounced abortifacient effects in rats [29]. Root powder of *Derris brevipes* at dose level of 200 and 600 mg/kg body weight showed 50% anti-implantation activity and also a significant reduction in the number of litters born and the ethanolic extract exhibited

Alcoholic extract of *Neem* flowers alters the estrous cycle, by prolonging the duration of the diestrus phase and subsequently lowering the frequency at which the estrus phase occurs. Consequently the frequency of ovulation is reduced and fertility may therefore be impaired. [30,31,32,33,34,35] In the present study of aqueous, ether, and chloroform extract of *Caesalpinia Pulcherrima* at 400 mg/kg body weight shows the antifertility effect of prolongation of estrous cycle and diestrus phase particularly in experimental animals. .The results of our study (Table 1) confirm the reports of the ability of some plant extracts to prolong the oestrus cycle and diestrus phase of the cycle [36, 37]

In the present study, *Caesalpinia pulcherima* (arial part) treated rats showed a decrease in the duration of proestrus, estrus and metestrus, while it increased the duration of diestrus. This is suggestive of negative influences on the estrous cycle as this reduces the number of days/ova ovulated during the proestrus and estrus phases.

In the present study, the doses 400 mg/kg body weight of caesalpinia pulcherima arial part shows a non significant change in the body weight of experimental animals but there was a significant decrease in the weight of the ovary and increased in the uterine weight in the treated rat. Reduction in the weight of the ovary and increased in the uterus after treatment of the animal with *caesalpinia pulcherima* arial part may be attributed to the absence or reduced availability of ovarian hormone and gonadotropine respectively. Data revealed that oral administration of steroidal fraction of fenugreek seed extract to female rats for fifteen days brought about a decrease in the weights of reproductive organs, indicating that the level of estrogen was not enough to

maintain the weights of reproductive organs. The structural and functional integrity of reproductive tissues depend on the circulating level of estrogen and therefore any small change in estrogen level may result in reduction in the weights of the reproductive organs. [38].

## 5. CONCLUSION

In conclusion, all the three extract i.e. aqueous, alcoholic and petroleum ether extract of *caesalpinia pulcherima* when administered orally possess antifertility activity. However other extract at 400mg/kg body weight showed 100% abortifacient activity. Further studies to identify the bioactive principle of abortifacient activity of the extract are in progress.

**Table 5.** Effect on estrous cycle of female albino rats after the administration of 400 mg/kg aqueous, ether, and chloroform extract of *Caesalpinia Pulcherrima*

Phases	Proestrous phase (days)	Estrous phase (days)	Metaestrous phase (days)	Diestrous phase (days)	Estrous cycle (days)
Vaginal opening/ cell type obtained in a vaginal smear	25% to 40% / Epithelial cells only	Above 70% / Few cornified cells	50% to 70% / Cornified cells plus many leukocyte	50% to 70% Leukocytes plus epithelial cells	
Group- A- (Control)	0.59±0.01	0.65±0.01	0.59±0.01	0.65±0.01	4.42±0.68
Group-B (Aqueous Extract 400mg/kg)	0.50±0.02***	0.62±0.02	0.83±0.01	2.67±0.03***	4.61±0.04***
Group-C ( ether Extract 400 mg/kg)	0.41±0.01***	0.55±0.01***	0.75±0.01****	3.30±0.03***	5.00±0.68***
Group-D (chloroform Extract 400mg/kg)	0.31±0.01***	0.54±0.03**	0.62±0.01***	3.92±0.03***	5.40±0.04***

Values in Mean±S.E. (Standard error), n=6, \*P<0.05, \*\*P<0.01, \*\*\*p<0.001, when compared with control.

**Table 6.** Body and organ weight changes in female albino rats after the administration of 400 mg/kg with aqueous, ether, chloroform extract of *Caesalpinia Pulcherrima* (arial parts)

Treatment Groups	Dose mg/kg	Body weight (gm)		Reproductive organ weight (mg)	
		Initial	Final	Ovary	Uterus
Group I- Control	Vehicle	147.16 ± 3.96	155.16 ± 4.42	28 ± 1.23	112 ± 1.42
Group II Aqueous extract	400 mg/kg	160.66 ± 0.85	168.33 ± 0.42***	24.83 ± 1.19	93.5 ± 1.43***
Group III Ether extract	400 mg/kg	151 ± 1.33	160 ± 0.85***	24.33 ± 0.88**	87.66 ± 1.87***
Group IV Chloroform extract	400 mg/kg	167.33 ± 1.68	175 ± 1.20***	21.2 ± 0.28***	69 ± 1.39***

Values in Mean±S.E. (Standard error), n=6, \*P<0.05, \*\*P<0.01, \*\*\*p<0.001, when compared with control.

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## Antifertility Effect of Aqueous, Ether and Chloroform Extract of *Caesalpinia Pulcherrima* on Female Albino Rats

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