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Antifertility activity of *Artabotrys odoratissimus* Roxb. and *Couroupita guianensis* Aubl.

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Abstract

Objective: To study antifertility activity of various extracts of *A. odoratissimus* Roxb. and *C. guianensis* Aubl. Materials and methods: Antifertility activity of benzene, ethanol and water extracts of leaves of *A. odoratissimus* and bark and flowers of *C. guianensis* was studied for their effect on duration of various stages of oestrus cycle in adult female rats and on the number implantation sites in the pregnant rats. Results: All the extracts of *A. odoratissimus* leaf and ethanol and water extracts of *C. guianensis* bark and flower showed significant prolongation of dioestrus stage ($p<0.05$). Ethanol and water extracts of *A. odoratissimus* leaf showed anti-implantation effect. Ethanol extract of *C. guianensis* bark and all the extracts of its flower reduced the number of implantations. Conclusion: The disturbances in the reproductive cycle indicate effect of these plants on the sex hormones in the animals. Prevention of implantation may be due their interference in the receptive stage of the uteri and endometrial sensitivity for decidualisation. The results suggest that the both plants are having antifertility activity and possible therapeutic use.

1. Introduction

In search of antifertility agents from plant origin, indigenous to India, two plants were selected from the literature [1, 2], namely *Artabotrys odoratissimus* Roxb. (Annonaceae) and *Couroupita guianensis* Aubl. [3]. *A. odoratissimus*, (Synonym: *A. Hexapetalus*) is locally known as Nag Champa [4] and was identified as a potential antifertility agent [5]. In India it is known for its antifertility effect [6]. Literature survey revealed that systematic study on this plant for the antifertility activity is still lacking. *C. guianensis* Aubl. is widely planted for its ornamental value. It is known as cannon ball tree in English and Kailaspati in Hindi and it belongs to the family Lecythidaceace [3,7].

The petroleum ether and methanol extracts of aerial parts were reported to possess antibacterial, antimalarial and anthelmintic activity [8]. The aqueous extract of the flower

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and bark are used by the folklore of Gujarat for prevention of conception. However, there is hardly any scientific report on its antifertility activity. Hence to ascertain above claims, we have taken up a systematic study on *A. odoratissimus* and *C. guianensis.*

2. Materials and methods

2.1 Plant material

Matured leaves of *A. odoratissimus* and barks and flowers of *C. guianensis* were collected from the botanical garden of Gujarat Agriculture University, Anand in the month of March-April. Comparing the herbarium available in the Dept. of Bioscience, Sardar Patel University, Vallabh Vidyanagar authenticated the plants and a voucher specimen of each plant part was submitted to the Department of Pharmacognosy, A. R. College of Pharmacy and G.H. Patel Institute of Pharmacy, Vallabh Vidyanagar.

2.2 Preparation of extracts

The plant materials were shade dried and milled to obtain coarse powder. The powdered materials were successively extracted with benzene and ethanol [95%] in a Soxhlet's apparatus using continuous hot percolation method followed by extraction with water. The individual extracts were collected and concentrated by evaporation in vacuum. The dried extracts were formulated as suspension using Tween 80 (5% w/v) as suspending agent. Various extracts of each plant material were evaluated for their effect on oestrus cycle and implantation.

2.3 Animals

Adult female albino rats of Wistar strain each weighing 100-125 g that were maintained under standard environmental conditions (25-28°C and 12 h light/dark cycle) were used. They were allowed standard laboratory feed and water at libitum.

2.4 Effect on oestrus cycle

The vaginal smear examination was done microscopically using 5% aqueous solution of methylene blue to check estrus cycle every morning at 08.00-10.00 h. The institutional animal ethical committee approved the experimental protocols. Different stages of oestrus cycle were determined according to WHO protocol [9]. Three consecutive cycles were studied and the rats, which showed normal oestrus cycle, were chosen for the study.

These rats were then segregated into ten groups of six animals each. Group 1, 2 and 3 were given benzene, alcohol and water extract of *A. odoratissimus* leaf respectively. Group 4, 5, and 6 were given benzene, alcohol and water extracts of *C. guianensis* bark and group 7, 8 and 9 received benzene, alcohol and water extracts of *C. guianensis* flower respectively. Group 10 received vehicle Tween 80 (5% w/v). The extracts were administrated orally at the dose of 250 mg/kg after taking the vaginal smears.

Three consecutive oestrus cycles were studied and the duration of each stage of the cycle were noted. The results were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnet's test for comparison of treated group with control group.p-values, <0.05 were considered statistically significant. (Table1)

2.5 Anti-implantation effect

Female rats of proven fertility were caged individually except during mating. When nucleated epithelial cells were observed in the vaginal smears, the female rats were paired with male rats of proven fertility in 3:2 (Female: Male) ratio for overnight.

Presence of copulation plugs or spermatozoa in the vaginal smear on the following morning was regarded as day one of pregnancy. Pregnant rats were segregated in ten groups of six animals each and extracts were

Table 1
Effect of various extracts on three consecutive oestrus cycles in rats. (Mean duration ± SEM in days)

Extracts	No. of days	Oestrus stage	Dioestrus stage	Pro-estrus stage	Meta-oestrus stage
A. odoratissimus Leaf					
Benzene	14.6 ± 0.58	2.7 ± 0.14	7.2 ± 0.24*	2.0 ± 0.16	2.7 ± 0.08
Alcohol	15.6 ± 0.18	2.4 ± 0.12	9.0 ± 0.45*	1.6 ± 0.39	2.6 ± 0.03
Water	15.7 ± 0.21	2.8 ± 0.02	8.6 ± 0.23*	1.7 ± 0.23	2.6 ± 0.12
C. guianensis Bark					
Benzene	14.5 ± 0.17	3.0 ± 0.12	6.5 ± 0.12	2.4 ± 0.13	2.6 ± 0.72
Alcohol	14.8 ± 0.71	2.2 ± 0.14	8.6 ± 0.10*	1.5 ± 0.15	2.5 ± 0.33
Water	14.7 ± 0.64	2.5 ± 0.18	7.5 ± 0.50*	2.5 ± 0.86	2.4 ± 0.35
C. guianensis Flower					
Benzene	14.0 ± 0.82	3.0 ± 0.62	6.2 ± 0.56	2.5 ± 0.68	2.6 ± 0.82
Alcohol	15.9 ± 0.64	3.1 ± 0.11	9.5 ± 0.13*	2.0 ± 0.16	2.5 ± 0.24
Water	15.0 ± 0.86	2.6 ± 0.43	8.0 ± 0.63*	2.3 ± 0.92	2.1 ± 0.15
Control	14.8 ± 0.33	3.3 ± 0.85	6.2 ± 0.18	2.5 ± 0.48	2.7 ± 0.12

* $p < 0.05$ Significantly different from the control when one way ANOVA coupled with Dunnet's test

administered similar to the effect on oestrus cycle. Laparotomy was performed on eleventh day of pregnancy under anesthetic ether and total number implantation sites in two horn of the uterus were counted to check the absence of implantation.

After the operation, wound was sutured and dressed with antibiotic powder every 6 h. The animals were then returned to their respective cages and allowed to deliver the litters. After parturition, the numbers of litters delivered were counted to check the reduction in implantation. The animals were observed carefully during the experiment for any deformities in the litters.

The results were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnet's test for comparison of treated group with control group. p-values, <0.05 were considered statistically significant. (Table 2)

On the basis of observed data, the antifertility activity was calculated in terms of percentage depending on the missing number of implantation by the total number of animals treated on laparotomy.

3. Results

The effect of various extracts on oestrus cycle and on the pregnancy of rats is presented in table 1 and 2. From the table 1 it is clear that all the extracts of *A. odoratissimus* leaf shows significant prolongation of dioestrus stage ($p<0.05$). Alcoholic extract of *A. odoratissimus* leaf increased the dioestrus stage to greater extent when compared to benzene and water extracts. Alcoholic and water extracts of *C. guianensis* bark and flower significantly prolonged the dioestrus stage ($p<0.05$).

From the table 2 it is clear that the alcohol and water extracts of *A. odoratissimus* leaf showed anti-implantation effect and the latter was found to be more effective. Alcohol extract of *C. guianensis* bark and all the extracts of its flower reduced the number of implantations. Water extract of *A. odoratissimus* leaf and benzene extract of *C. guianensis* flower showed maximum activity (83.3%).

Table 2
Effect of extracts on pregnancy in rats (treatment day 1-10 days)

Extracts	Number of implantation sites / number of rats treated	Number of Implantation Mean ± SEM	Average Litters delivered Mean ± SEM	Antifertility activity (%)
A. odoratissimus Leaf				
Benzene	3/6	4.3 ± 0.2	4.2 ± 0.25	50
Alcohol	2/6	3.5 ± 0.5*	2.3 ± 0.50	66.6
Water	1/6	2.2 ± 1.2*	1.8 ± 0.75	83.3
C. guianensis Bark				
Benzene	3/6	8.2 ± 2.8	8.7 ± 0.2	50
Alcohol	2/6	3.2 ± 1.2*	3.8 ± 1.2	66.6
Water	3/6	7.8 ± 0.7	7.5 ± 0.7	50
C. guianensis Flower				
Benzene	2/6	2.4 ± 0.6*	0.5 ± 0.3	66.6
Alcohol	1/6	0.8 ± 0.1*	1.5 ± 0.2	83.3
Water	2/6	2.4 ± 0.5*	1.4 ± 0.2	66.6
Control	6/6	10.2 ±3.2	9.8 ±1.9	--

* $p < 0.05$ Significantly different from the control when one way ANOVA coupled with Dunnet's test.

4. Discussion

Oral administration of *A. odoratissimus* and *C. guianensis* extracts prolonged the dioestrus stage (table-1) and oestrus cycle was also disrupted. Ethanol and water extracts of *A. odoratissimus* leaf, ethanol extract of *C. guianensis* bark and all the extracts of its flower reduced the number of implantations (table-2).

Oestrus cycle is under the control of ovarian hormones, oestrogen and progesterone, secreted by the cells of membrana granulosae of the matured follicle and the corpus luteum respectively which, in is turn regulated by gonadotropins of the pituitary. These hormones also govern the shift of various stages of oestrus cycle.

The present study revealed that the extracts disturb the oestrus cycle by extending the dioestrus stage. Anti implantation may be due to the interference in the receptive stage of the uteri and endometrial sensitivity for the decidualisation. But at this stage anti-oestrogenic property cannot be attributed to these extracts as the study was performed on the normal rats.

5. Conclusion

Our findings revealed that the extract contain a property which disrupts the oestrus cycle and extends the dioestrus stage. It can be considered as an anti fertility property but at present cannot confirm it is due to particular hormone. The anti implantation potential of the extracts and the chemical constituent will be studied to document the active constituents responsible for the effect. Further work to elucidate its mode of action and the possible therapeutic use of the plants is under progress.

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