Antifertility Effect of *Cyclea Burmanni*

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The petroleum ether and chloroform extracts of the roots of *Cyclea burmanni* (Fam. Menispermaceae) have been found to possess significant antifertility effect in rats. Both these extracts exhibited partial and complete resorption of implants at 200 and 400 mg/kg body weight dose levels. In estrogenic activity study, both the extracts increased uterine weight and caused opening and cornification of vagina in immature rats. The present work justifies its effectiveness in preventing pregnancy in all rats when administered at 400 mg/kg p.o.

Search for the antifertility factor in plants remains a potential area of investigation. *Cyclea burmanni* (Fam. Menispermaceae) is known for its several medicinal uses like anthelmintic, antiinflammatory, febrifuge, diuretic and tonic[1]. The roots are useful in cough, bronchitis, dyspepsia, ulcers, wounds, diarrhoea, dysentery, fever, vomiting, burning sensation, pruritus, worms, respiratory and cardiac disorders[2-4]. The decoction of the roots is reported to be used to treat hemorrhoids and stomach pain[5]. The tribes of Ganjam district of Orissa use the fresh root juice orally to induce abortion. An extensive literature survey available from all scientific sources revealed no information about the pharmacological validation of the antifertility activity of the plant. The present work was therefore undertaken to substantiate the folklore claims in a scientific manner using animal models.

The plant material was collected from Hinjilicat of Ganjam district of Orissa and identified by the taxonomists of Botany Department of Utkal University, Bhubaneswar by comparing with the voucher specimen present in the department herbarium. After authentication, the plant material was collected in bulk, washed under running tap water to remove adhering dust, dried under shade and ground down through a grinding mill. The resulting coarse powder was extracted successively with petroleum ether (60-80°) and chloroform in a Soxhlet apparatus. The liquid extracts (yield: petroleum ether extract 1.44% w/w and chloroform extract 1.49% w/w with respect to dried material) were concentrated under vacuum to near dryness. The test samples were prepared by making an emulsion using gum acacia.

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Antifertility testing was performed on adult female Wistar rats weighing between 150-200 g were obtained from the animal house of Institute of Pharmacy and Technology, Salipur. The animals were maintained in acrylic cages at room temperature with standard pellet diet and water *ad libitum*. That the rats used in the experiments were fertile was first ascertained by breeding these virgin females with males of proven fertility. The female rats were used for the tests only after they had borne one or two litters, proving their fertility. Estrogenic activity of the extracts was assessed in immature female rats.

The antifertility study was performed as suggested by Thompson[6] and Khanna *et al*[7]. The experimental protocols have been approved by the Institutional Animal Ethics Committee. The vaginal smears of such female rats of known fertility were examined daily and the rats in proestrous phase of the estrous cycle were left overnight with known fertile males. The female rats were examined the following morning for evidence of copulation. Those rats, which showed thick clumps of spermatozoa in their vaginal smears, were separated from the experiment and the day the spermatozoa were found was labeled as day 1 of pregnancy. Mated rats were randomly distributed into various groups of five animals in each. The extracts to be tested were fed orally to these pregnant rats at a dose of 200 and 400 mg/kg twice

TABLE 1: EFFECT OF VARIOUS EXTRACTS OF *C. BURMANNI* ON BILATERALLY OVARIECTOMIZED IMMATURE RATS.

Treatment	Dose mg/kg	Uterine weight (mg)	Vaginal opening (%)	Vaginal cornification (%)
Control (Vehicle)	-	101.8±6.9	0	0
Diethylstilbestrol	1.5	202.7±10.1*	100	90
Pet. ether extract	400	211.6±10.4	100	100
Chloroform extract	400	196.7± 8.9*	90	90

Results expressed as Mean±SEM n=6, All p values are calculated with respect to vehicle control, *denotes statistical significance at $p<0.01$. In case of vehicle control, the dose administered was 2 ml/kg.

daily through an intragastric catheter. The control group received only vehicle (1%w/v gum acacia) in a similar manner. Treatment was given for 10 d and the animals were laporotomised under mild ether anesthesia on day 16. The 2 horns of the uterus were examined for the number of implants and prominent corpora lutea.

Estrogenic activity of the extracts was assessed in immature female rats as suggested by Zarrow *et al.*[8]. The selected rats were bilaterally ovariectomized under mild ether anesthesia through lateral incisions in the skin just below the last rib. The ovariectomized rats were divided into three groups of control, standard and test respectively. The different groups of animals received one of the following through oral route: vehicle (2 ml/kg), diethylstilbestrol (1.5 mg/kg), petroleum ether extract (400 mg/kg) or chloroform extract (400 mg/kg) once daily for a period of 5 d. After 24 h of last dose treatment, the animals were sacrificed and uteri were excised from adhering tissue and weighed. Vaginal opening and vaginal cornification were also recorded (Table 1). Statistical analysis of the differences observed between control and treated groups were carried out wherever applicable using Student's *t*-test. The level of significance for all determinations was $p<0.01$.

The antifertility effect of the petroleum ether and chloroform extracts together with dose, the number of implantation sites and number of corpora lutea are shown in Table 2. The results clearly showed that both petroleum ether and chloroform extracts possess antifertility effect in a dose-dependant manner. However, the petroleum ether extract is found to be more active than the chloroform extract as 60% activity was observed with 200 mg/kg dose of petroleum ether extract in place of 40% activity with chloroform ex-

TABLE 2: EFFECT OF VARIOUS EXTRACTS OF *C. BURMANNI* ON IMPLANTATION IN RATS.

Nature of extract	Dose mg/kg	Sample size	Animals showing implantation no. of implants	% of rats with no implantation sites	Number of corpora lutea
Vehicle	-	5	5/10,11,10,9,12	0	14,11,14,10,11
Petroleum ether	200	5	2/3,3	60	4,3
	400	5	0	100	NIL
Chloroform	200	5	3/4,3,3	40	4,4,3
	400	5	0	100	NIL

Data shows the number of implants and the number of corpora lutea on Day 16 of the study. In case of vehicle, the dose administration was 2 ml/kg.

tract. Resorption of implants was observed with 400 mg dose of both the extracts on laparotomy, as evidenced by scar marks of implantation sites in the uterine horns of animals. In animals treated with 200 mg dose for both the extracts, on laparotomy the uterine horns showed reduced number of implantation sites compared to the control group animals.

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 d of age[9]. Reproductive and general metabolic effects in mature and immature rats are manipulated with the ingestion of phytoestrogenic substances and produce effects similar to that of gonadal steroid 17 β–estradiol[10]. A number of plant extracts have been shown to exhibit estrogenic activity in rats[11]. In the present investigation, the root extracts have also shown a prominent estrogenic activity as evidenced from Table 1. Both the extracts increased uterine weight and caused opening and cornification of vagina in immature rats. Preliminary phytochemical observations of the petroleum ether extract reveals the presence of steroids and that of chloroform extract reveals the presence of steroids and alkaloids which could be possibly responsible for the activity. The roots of *Cyclea burmanni* are reported to have contraceptive value in the folklore remedies. The present work justifies its effectiveness in preventing pregnancy in all rats when administered at 400 mg/kg p.o.

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Spectrophotometric Method for the Estimation of Cefpodoxime Proxetil

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A simple and reproducible spectrophotometric method has been developed for the estimation of cefpodoxime proxetil. This method is based on the reaction of the drug with ferric chloride and potassium ferricyanide, which forms a green chromogen exhibiting maximum absorption at 780 nm.

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