



**EFFECT OF CYNODON DACTYLON EXTRACT ON ESTROUS CYCLE AND REPRODUCTIVE
ORGANS IN FEMALE WISTAR RATS**

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ABSTRACT: Population explosion is a major problem which might affect drastically on economic growth. Although, synthetic contraceptive are available, these cannot be used continuously due to their side effects. Thus the present was to evaluate the effect of aqueous extract of entire plant of cynodon dactylon extract on reproductive organ weight and estrous cycle in female rats. Wistar female rats were orally administered with aqueous extract of entire plant of cynodon dactylon (400 mg/kg body weight /day, for 30 days) . Results of the present study showed a significant increase ($P<0.001$) in the weight of the uterus and a significant decrease ($P<0.001$) in the weight of the ovaries was observed in the treated group. Further, the estrous cycle was found to be irregular and disturbed. In conclusion, the present study suggests that the aqueous extract of entire plant of cynodon dactylon possess antifertility activity

Key words: Cynodon dactylon, Estrous cycle, Ovary, Uterus, Antifertility

INTRODUCTION

Plant materials continue to play an important role in the maintenance of human health since antiquity. Over 50% of all modern chemical drugs originated from natural plant sources [1]. These plant products are the major source of drug development in pharmaceutical industry [2]. Rural dwellers in most parts of the world do not depend on the orthodox medicine for the cure of diseases and ailments [3]. This is because most of the modern equipment's are expensive and service delivery too expensive to afford. As a result of this, a larger section has resulted to the use of traditional medicines, which are believed to be less expensive, and of little or no side effects.

Control of population growth is very important in populated countries. Current methods of contraception result in an unacceptable rate of unwanted pregnancies and having side effects also [4,5,6]. Therefore, the screening of plants with antifertility activity and the subsequent identification and characterization of the active components will be a useful guide towards the formulation of cheaper, affordable contraceptive with reduced toxicity. Numerous plants have been reportedly used historically by women to aid child delivery, stimulate menstrual flow or reduce fertility [7,8]. Modern scientific studies in experimental animals have confirmed the effect of some of these herbs on the reproductive system without producing apparent toxic effects [9,10]. *Cynodon dactylon Pers.* (Family: Graminae, Durba in Bengali, Dhub in Hindi, Bermuda grass in English), a creeping grass found in warm climates all over the world between 450 south and north altitude. The *Cynodon dactylon* is available throughout the year; the material is used by the domestic animals as food and for pooja in all parts of India.

The juice of the plant is astringent and is applied externally to fresh cuts and wounds. It is also useful in treatment of catarrhal ophthalmia, dropsy, hysteria, epilepsy, insanity, chronic diarrhea and dysentery [11]. The plant is folk remedy for anasarca, calculus, cancer, carbuncles, cough, hypertension, snakebites, stones, gout, fever, skin diseases and rheumatic infections. It has also antioxidant properties, CNS depressant activities as antidiabetic, antiviral agent. The rhizome is used as anti-inflammatory, diuretic, antiemetic, purifying agent and also in dysentery [12,13]. Despite these traditional claims, no in depth scientific study has been performed regarding its antifertility effect of entire plant on female reproduction. The present work was designed to evaluate the antifertility potential of aqueous extracts of entire plant of *Cynodon dactylon* in female wistar rats.

MATERIAL AND METHODS

Plant material ^[14]

The whole plant with the roots of *Cynodon dactylon* was collected from the campus of Kasturba Medical College, Manipal University, Mangalore, India. 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. The dried 20 g plant were powdered and soaked separately in 100 ml water and chloroform by keeping it in a shaker for 3 days. Extracts were filtered through cheesecloth and the extracts were reduced to 10% of its original volume. The organic solvent filtrates were concentrated in vacuum using a rotary evaporator, while aqueous extract was dried using water bath. The extract preparation for the present experiment was done in Yenopoya Medical College.

Acute oral toxicity study

Acute toxicity study was carried out as per prescribed Organization for Economic Cooperation and Development guidelines. Prior to experimentation animals (n=6) were fasted overnight (but not water withheld for 3-4 h) and was orally administered with fixed extracts dose of 50, 200, 400 and 2000 mg/kg body weight respectively by gavage using intubation canula. The dose was found tolerable as no death was found up to the maximum administered doses. Rats were observed individually after dosing for first 30 min periodically and daily thereafter, till 14 days for any toxicity sign of gross changes in skin and fur, eyes and mucous membranes, circulatory, respiratory, autonomic and central nervous systems, and behavior pattern if any. On the basis of earlier studies [14, 15] carried the effective dose 400 mg/kg was being selected for further studies.

Phytochemical screening: Chemical tests were carried out on aqueous extracts of *Cynodon dactylon* using standard procedures to identify the constituents as described by Sofowora [16]. Trease and Evans [17] and Harborne [18].

Alkaloids: About 0.2 g of the extracts was warmed with 2% H₂SO₄ for two minutes. It was filtered and few drops of Dragendorff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

Tannins: Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Steroids: 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Phlobatanins: The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl. Red precipitate shows the presence of phlobatanins.

Flavonoids: Extract of about 0.2 g was dissolved in NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

Saponins: About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing of the extracts shows the presence of saponins.

Experimental Animals

Twelve female albino rats (Wistar strain) weighing between 120–130 g body weight were obtained from the animal house of Kasturba Medical college (Manipal university) Mangalore. They were housed in the institutional experimental animal laboratory. The rats were kept in cages in a room maintained at 26–29 °C with a 12-hour light-dark cycle for 4-weeks to acclimatize, and were allowed free access to food and water ad libitum. All the rats received approximately 20 gms of standard rat pellets (Lipton, India Ltd. Bangalore) per day, with or without cholesterol added. All the animal procedures were carried out in strict compliance with the institutional animal ethical committee regulations.

Experimental design

The effect of *cynodon dactylon* plant extract on normal and treated rats were studied. All the rats received treatment for 30 days. The rats were randomly distributed into two groups of six animals each. Group I served as a control rats (administered with 0.5ml distilled water) and Group II served as a treated groups to be given *cynodon dactylon* at a dose of 400 mg/kg body weight.

Estrous cycle evaluation [19]

Daily vaginal smears has to be performed every morning in between 8:00 and 9:00 a.m. Vaginal secretion has to be collected with a plastic pipette filled with 10 ml of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply. Vaginal fluid are to be placed on glass slides. A different glass slide has to be used for each animal. One drop was collected with a clean tip from each rat. Unstained material was to be observed under a light microscope, without the use of the condenser lens, with 10 and 40x objective lenses. A normal estrous cycle in rats was defined as 4–5 days. Three types of cells to be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. The proportion among them was used for the determination of the estrous cycle stages. The estrous cycle stages are (1) proestrus (mainly epithelial cells), (2) estrus (mainly cornified cells), (3) metestrus (cornified and leukocytes), and (4) diestrus (mainly leukocytes) present. Animals showing normal and regular estrous cycle (4 to 5 days) for at least three consecutive times were selected for the experiment. Cycles were considered prolonged if the rat remained in one phase for 4 days and acyclic if rat remained in one phase for less than 15 days.

Body weight and organ weight [20]

Body weight was determined just before killing of each animal. The rats of both the groups were sacrificed by administering sodium pentobarbitone; 40mg/kgBW on the 31st day. After sacrificing the animal, an incision is made in the abdomen, the uteri along with the ovaries were removed. Fat and connective tissue if any was trimmed and weighed immediately (wet weight) using a sensitive electronic balance. The relative ovarian and uterine wet weight to body weight ratio was calculated for each animal by dividing the organ weight by body weight (bw) and multiplying by 100.

STATISTICAL ANALYSIS

The experimental results were expressed as Mean \pm SD data were assessed by the method of analysis of ANOVA followed by student t- test $p < 0.05$ were considered as statistically significant [21].

Table 1: Phytochemical Constituents of Aqueous Extract of Cynodon Dactylon

Phytochemical	Positive(+) / Negative (-)
ALKALOIDS	-
TANNINS	+
STEROIDS	+
PHLOBATANIN	-
FLAVINOIDS	+
SAPONINS	+

RESULTS

The phytochemical analysis of aqueous extract had shown the presence of tannins, steroids, flavinoids, saponins, and absence of alkaloids and phlobatanins (Table 1). Treated group rats showed a significant decrease ($P < 0.001$) in the duration of proestrous, estrous and metaestrus and significant increase ($P < 0.001$) in the duration of diestrus phase, when compared to control group (Figure 1). Further, A significant increase ($P < 0.001$) in the weight of the uterus and significant decrease in the weight of the ovaries ($P < 0.001$) was observed in the treated group when compared to the control group

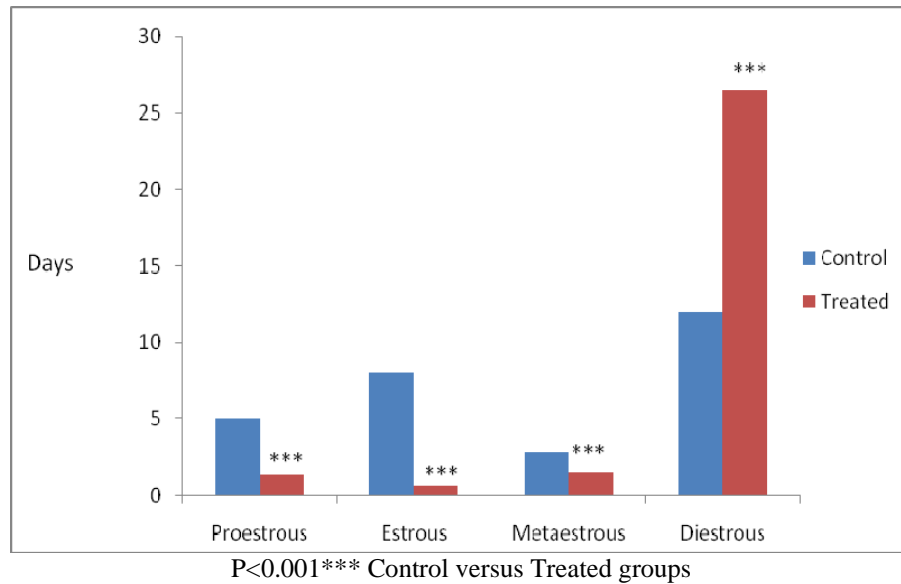


Figure 1: Effect of cynodon dactylon extract on estrous cycle in wistar female rats. “Values are expressed in Mean ±SD”.

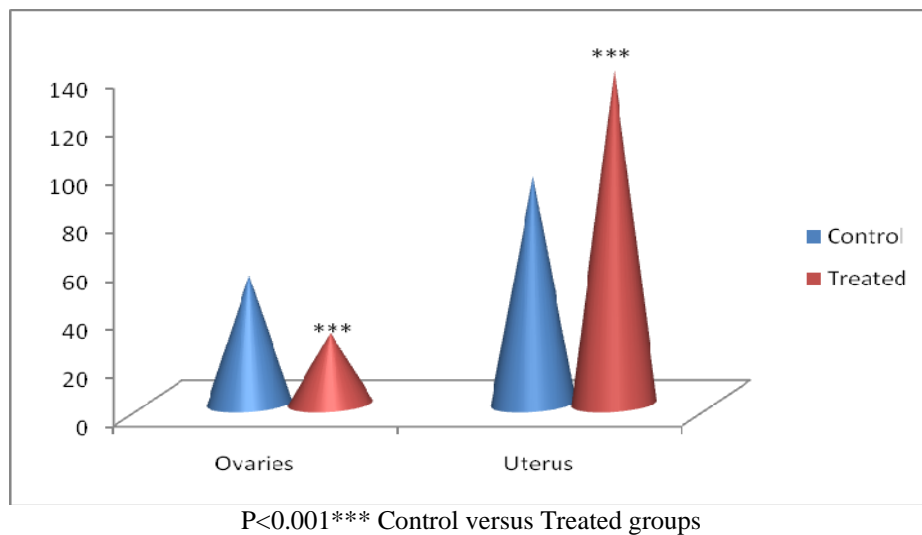


Figure 2: Effect of cynodon dactylon extract on weight of the ovaries and uterus in wistar female rats. “Values are expressed in Mean ±SD”.

DISCUSSION

Termination of pregnancy has been practiced since antiquity. Although synthetic abortifacients of known mechanisms are effective and popular, but the risks associated with these drugs have triggered the need to develop new molecules from medicinal plants, which stimulated our interest in knowing the antifertility effect of the entire plant of cynodon dactylon. Preliminary phytochemical studies of the extract indicated the presence of a tannin, flavonoid saponins, steroids which are reported to have contraceptive activity [22, 23]. The absence of clinical toxicity symptoms in the treated female rats such as tremors, weakness, and refusal of feeds, diarrhoea, weight loss, hair loss, coma and death suggests that the extract was not clinically toxic to the female rats.

Endocrine changes and decline in endocrine function involve tissue responsiveness, reduced secretory output from peripheral glands and alterations in the central mechanism controlling the temporal organization of hormonal release [24]. An estrous cycle is a rhythmic reproductive cycle in sexually matured female mammals and is influenced by the release of gonadotropin releasing hormone from the hypothalamus, gonadotropins from the pituitary gland and sex hormones from the gonads. While female cyclicity characterized by vaginal changes as observed in estrus cycle is an index of good functioning of the neuroendocrine – reproductive system and ovarian activity, loss of normal estrus cycle indicates the disruption of ovarian progesterone and estrogen balance [25, 26, 27]. The presence of particular cell types indicates the follicular and luteal phases of the reproductive cycle. Although we did not evaluate hormonal milieu in the present study, other researchers using vaginal smear to monitor the estrous cycle of albino rats also indicated an alteration of estrus cycle and disruption of ovarian endocrine function [28, 29]

In the present study, cynodon dactylon 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 influence on the estrous cycle as this reduces the number of days/ova ovulated during the proestrus and estrus phases. Presence of phytochemicals like tannin, flavonoid saponins, steroids in the cynodon extract might be a contributing factor for disruption of estrous cycle [30]. Estrogenic chemicals are known to cause infertility by shortening the time of transport of egg, disrupting estrous cycle, lowering the plasma progesterone and decreasing pregnanediol which finally stops development of endometrium [31, 32]. The variations observed in the reproductive organ weights in the treated rats might be attributed to phytoestrogenic components of the extract. Decrease in the ovarian wet weight might be associated with inhibition of release of pituitary gonadotropins due to negative feedback mechanism of phytoestrogens on the pituitary hormones.

Different plants have different mechanisms by which they alter the reproductive cycle. Based on the present results we presume that the estrogenic substance and phytochemical present in the cynodon extract may individually or synergistically affect the reproductive function. In conclusion, the present study suggests that the aqueous extract of entire plant of cynodon dactylon possess antifertility activity by causing the cessation of estrous cycle thereby altering the physiologic functions of the reproductive organs. Further studies are needed to prove whether the alterations are reversible or permanent after cessation of treatment and for understanding the exact mechanism.

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