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EFFECT OF AQUEOUS EXTRACT OF CYNODON DACTYLON ON REPRODUCTIVE HORMONES AND REPRODUCTIVE ORGAN WEIGHT OF FEMALE WISTAR RATS

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ABSTRACT

Plant-derived chemicals have its profound influence on endocrine activities in both humans and animals. The increase in the rate of infertility in women has prompted the need to search for plants with antifertility potentials, devoid of harmful side effects.The present study focused on the effect of administration of aqueous extract of entire plant of cynodon dactylon forthirty days on reproductive hormones and reproductive organ weight of female Wistar rats .Phytochemical screening of the extract revealed the presence of tannins, steroids, flavinoids, saponins .Administration of the extract produced significant increase ($P<0.001$) in the serum estradiol concentration whereas those of, follicle stimulating and luteinizing hormones were significantly ($P<0.001$) reduced.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.In conclusion the present study suggest that the aqueous extract of cynodon dactylon might contains biologically active phytochemicals which might cause disturbance in the female reproductive physiology.

Keywords: Cynodon Dactylon, Follicle Stimulating Hormone, Luteinizing Hormone, Estradiol, Antifertility and Contraceptive

INTRODUCTION

Population explosion is an imminent hurdle for a country's development as the natural resources are limited. Fertility regulation has therefore become the major concern of people of all walks of life. Plant preparations play an important role in ancient literature of indigenous systems of medicine **[1**, **2]**. A number of plant species have been tested for fertility regulation beginning about 50 years ago and were subsequently fortified by national and international agencies **[3**, **4]**. Plant-derived chemicals have its profound influenceon endocrine activities in both humans and animals. Previous studies focus on the antifertility effect of the plants mediating their action on hypothlamopituitary- gonadal axis or direct hormonal effects on reproductive organs resulting in inhibition of ovarian steroidogenesis **[5**, **6]**.Several studies have shown that chemical compounds including plant extracts could alter the concentrations and functions of female reproductive hormones **[7**, **8]**. In recent years, plants are pursued over steroidal contraceptive drug because plants are easily available, economic and devoid of harmful side effects.

The cynodon dactylon (Family; Poaceae), commonly known as "doob" (Hindi),aroogumpillo‟ (Tamil), is called creeper in India, known to be a tackler in Indian mythology and is offered to Lord Ganesha. Leaf, root and rhizome of the plant havebeen used in folk medicine in different countries, as anti-inflammatory, anticystitis **[9]**, antihypertensive, antiviral, hypolipidemic agent, antihysteria,antipsychotic, and antigonorrheal infection **[10]**. InIndia, the plant is used in the treatment of melena,thirst, anorexia, burning sensations in the body,pruritis, miscarriage and erysipelas and its leafjuice with a pinch of common salt has been usedorally to treat stomachache **[11**, **12]**. Decoction of wholeplant is given orally to cure menstrual problem **[12]**. However, little information is available on its effect onreproductive hormones of the females. The present study was therefore designedto provide information on the effect of aqueousextract of entire plant of cynodon

dactylon leaves on femalereproductive hormones and reproductive organ weight.

MATERIAL AND METHODS

Plant Material

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 **[13]**.

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 oral 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 **[13, 14]** 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 **[15]** Trease and Evans **[16]** and Harborne **[17]**.

Alkaloids

About 0.2 g of the extracts was warned with 2% $H_2S04$for two minutes. It was filtered and few drops of Dragencloffs 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 H_2 $S0_4$.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.

Body Weight and Organ Weight

Body weight was determined just before killing of each animal. The rats of both the groups were sacrificed by administering sodium pentobarbiotne; 40mg/kg BW 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 **[18]**.

Preparation of Serum

The rats were sacrificed by administering sodium pentobarbiotne; 40mg/kgBW. Blood samples were collected directly via cardiac puncture using 23G needles 5 ml of the blood was collected into clean and dry centrifuge tubes. The blood was then left for 10 min to clot at room temperature.The tubes were thereafter centrifuged at 33.5 g x 15 min using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals and Essex,England). The sera were later aspirated with Pasteur pipettes into clean, dry, sample bottles and were then used within 12 h of preparation for the hormonal assay.

Hormonal Assay

The procedure described in the hormone assay kits was used according to the principle highlighted by Tietz **[19]** for estradiol while that of Uotila *et al.*, **[20]** was used for luteinizing and follicle stimulating hormones.

Statistical Analysis

Results were expressed as the mean of six replicates±SD except for the phytochemical screening. Means were analyzed using a one-way ANOVA and values at $p<0.05$ were considered statistically significant **[21]**.

RESULTS

The phytochemical analysis of aqueous extract had shown the presence of tannins, steroids, flavinoids, saponins, and absence of alkaloids and phlobatanins **(Table 1)**. 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 **(Table 2)**.The concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH) was significantly ($P<0.001$) decreased in the treated group whereas, a significant increase ($P<0.001$)in the serum estradiol was observed in the treated group **(Figures 1-3)**.

Table 1: Phytochemical Constituients of Aqueous Extract of Cynodon Dactylon

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

Table 2: Effect of Cynodon Dactylon Extract on Weight of the Ovaries and Uterus in Wistar Female Rats ''Values are expressed in Mean ±SD"

PARAMETRS	CONTROL	TREATED
OVARIES	53.01± 1.33	29.3± 3.15***
UTERUS	94± 1.78	138± 4.38 ***

P<0.001* Control versus Treated Groups**

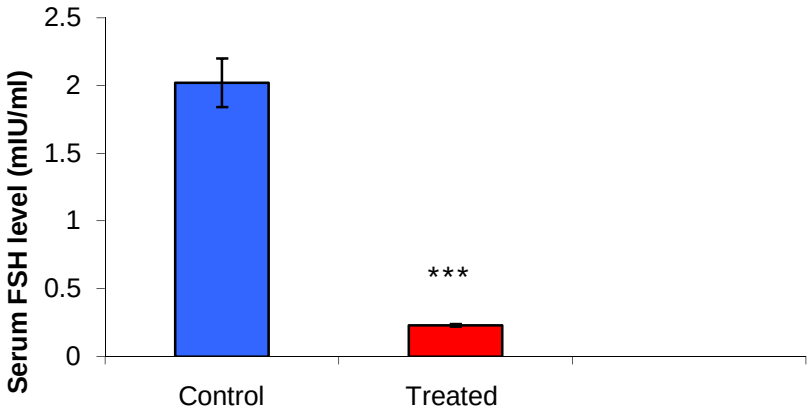

Figure 1: Effect of Cynodon Dactylon Extract on Serum FSH Level Inwistar Female Rats ''Values are expressed in Mean ±SD"; P<0.001* Control versus Treated groups**

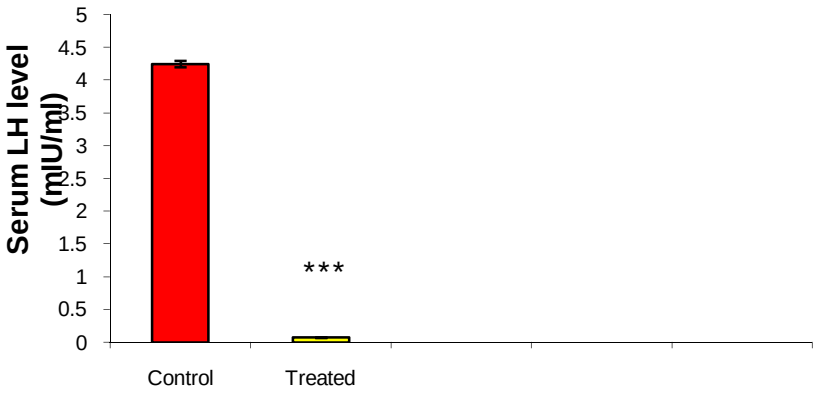

Figure 2: Effect of Cynodon Dactylon Extract on Serum LH Level Inwistar Female Rats''Values are Expressed in Mean ±SD".P<0.001* Control Versus Treated Groups**

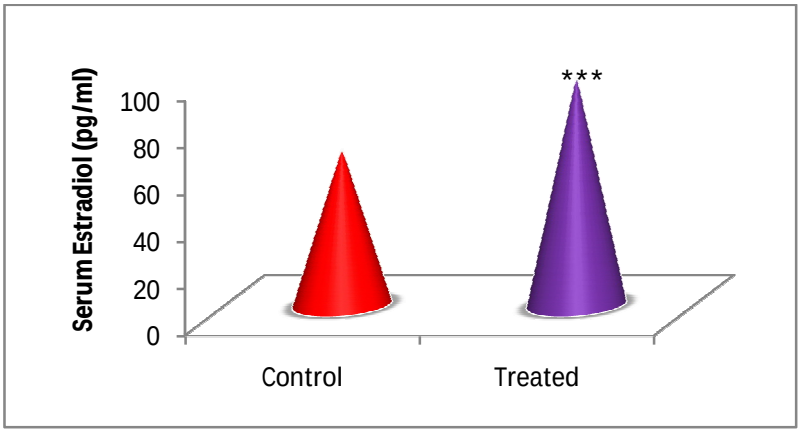

Figure 3: Effect of Cynodon Dactylon Extract on Serum Estradiol Level Inwistar Female Rats. ''Values are Expressed in Mean ±SD"; P<0.001* Control Versus Treated groups**

DISCUSSION AND CONCLUSION

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 **[22]**.The female reproductive activityis under the combined and balanced influences of ovarian and extra ovarian hormones **[23]**. Imbalances or alterations in these hormones lead to irregularity in the reproductive functions.Previous studies show that the hormonal imbalances are caused by numerous chemical agents contained in plant extracts **[24, 25]**.

Follicle stimulating hormone is the central hormone of mammalian reproduction, essential for gonadal development and maturation at puberty aswell as gamete

production during the fertile phase of life **[26]** It stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells. The reduction in the levels of FSH in the cynodon dactylon treated rats might hinder the process of folliculogenesis and delay maturation of the follicle **[27]**. Luteinizing hormone stimulates secretion of sex steroids from the gonads. In females, ovulation of mature follicles in the ovary is induced by a largesurge of LH secretion.Any substance capable of inhibiting this release could provokedisruption of ovulation by decreasing the number of mature follicles or induce an estrous cycle disruption at rest **[24]**. Therefore, the reduction in the serum LH levels may be explained by an inhibitory effect of the extract on the release of LHwhich may trigger disruption of ovulation process in treated female rats. Further, It is also possible that the extract might have exerted its effect on the anterior pituitary or the hypothalamus causing dysregulation in the hormonal secretion. Our study is in agreement with the previous plant extract studies which have also decreased the release of the gonadotropins (LH and FSH) **[24, 28]**.

Various parts of the different plants contain estrogens and they have their effects on the different life processes. Phytoestrogens are noble estrogens found in variety of plants, which may be ingested directly or as constituents of tissue from animals that have ingested plants. Phytoestrogens have noxious effect leading to impaired fertility in domestic animals as well as disturbance of the normal gestation process **[29, 30, 31]**. They may affect and regulate the reproduction and reproductive cycle. Estrogen acts in a feedback mechanism, influencing the production gonadotropins from the pituitary gland.Exogenous hormones exert a negative feedback on the hypothalamus in a manner similar to that by the naturally occurring hormones.Reports show that hypothalamic suppression by an oral contraceptive decreases the gonadotropic output from the pituitary **[32, 23, 24]**. Present findings indicate that the administration of the extracts showed significant increase in the estrogen level which might induce ainhibitory effect on gonadotropins. 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 in the present study is well associated with inhibition of release of pituitary gonadotropins due to negative feed back mechanism of phytoestrogens on the pituitary hormones.

Phytochemical screening has revealed many bioactive as well as toxic agents of plant extracts that can affect the regulation of reproduction causing hormonal imbalance. Flavonoids and saponins have been shown to reduce plasma concentrations of LH, estradiol and FSH **[32, 33]**. Therefore, it is possible that the aqueous extract of cynodon dactylon might contains biologically active phytochemicals which may be endocrine disrupting.The cynodon dactylon extract as concluded from the results elevated estrogenic activity, which (by feed back) inhibits FSH secretion from pituitary and therefore prevent the development of new follicle in ovary resulting inhibition of ovulation and impairment of fertility and thus contraception. Our preliminary findings in this study have importantimplications for female contraceptivedevelopment. Therefore, there remains definite scope for further research on cynodon dactylon as they provide safe contraception, without disturbing general physical conditions, as they did not show any toxic effect in present study.

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