Bharath
INSTITUTE OF HIGHER EDUCATION AND RESEARCH
(Declared as Deemed-to-be University under section 3 of UGC Act, 1956)
(Vide Notification No. F.9-5/2000 - U.3, Ministry of Human Resource Development, Govt. of India, dated 4th July 2002)
Accredited by NAAC
NATIONAL ASSESSMENT AND ACCREDITATION COUNCIL
Phone : 044-22290742 / 22290125 . Telefax : 044-22293886
Website : www.bharathuniv.ac.in
173, Agaram Road, Selaiyur, Tambaram,
Chennai - 600 073. Tamil Nadu.

Ref. No.SMS-2018-O-07

Date: 20.02.2019

TO

Mr. Anandaramajayan
Assistant Professor/Anatomy
BIHER

Thro: Concern Head of the Department

Greetings!!!

We are happy to announce that the Research Advisory Committee has approved your proposal for Seed Money Scheme-2018 which was presented by you. You are requested to complete the proposal and send the progress report to the Dean Research in the prescribed time period.

Title of the Project: Regulation of estrous cycle by Cynodon dactylon in letrozole induced polycystic ovarian syndrome in Wistars albino rats

Seed Money Amount: Rs.1, 00,000/- (Rupees One Lakh Only)

Approved on: 05.02.2019

Payment details:

Voucher No.52 **Dated: 27.02.2019**

With Regards

Dean-Research

Bharath University

SELAIYUR, CHENNAI - 600 073, TAMIL NADU, INDIA.

CASH / PAYMENT VOUCHER

Date 27/02/2019

V.No. 52

Debit _____ Amount

Rs.

PAID TO Dr. Anandaramajayan

RUPEES One Lakh only

TOWARDS Seed Money scheme – 2018

BHARATH UNIVERSITY CHENNAI 600 073. INDIA

Authorised by **Finance Manager** **Cashier/Accountant** **Payee's Signature**

PROPOSAL SUBMISSION

1. **Details of Principal Investigator**

Name	: N. Anandaramajayan
Designation	: Assistant Professor
Highest Qualifications	: M.Sc.,
Department	: Anatomy
E-mail	: anandaraman2006@gmail.com
Contact no	: 9894343517
Date of Joining	: 1-12-2010

2. **Details of Principal Investigator**

Name	: Dr. B Rajesh
Designation	: Professor
Highest Qualifications	: Ph.D.
Department	: Anatomy
E-mail	: anat_rajesh@rediffmail.com
Contact no	: 9345575143
Date of Joining	: 14.06.2010

Technical details

1. Introduction:

The menstrual cycle is a standard reproductive cycle that takes place in a female lifetime from the era of puberty to menopause. Two significant hormones control this menstrual cycle, namely follicular stimulating hormone and luteinizing hormone (LH), which are regulated by the release hormone of gonadotropin. Gonadotropin-releasing hormone of hypothalamus stimulates the follicle-stimulating hormone and LH in a pulsative frequency. It causes polycystic ovarian syndrome (PCOS) when this pulse frequency is disrupted [1-3]. PCOS is identified by Rotterdam criteria using the following signs and symptoms: (1) hyperandrogenism and/or hyperandrogenemia, (2) oligo-ovulation, (3) exclusion of known disorders, such as Cushing's syndrome, hyperprolactinemia, congenital adrenal hyperplasia and (4) polycystic ovaries on ultrasound [4-6]. Studies have been performed to treat PCOS using animal models for reasons such as shorter life span and differences in the estrous cycle, endocrine changes, and morphological similarities.

2. Review of status of Research and Development in the subject

Nallathambi A, Bhargavan R. Regulation of estrous cycle by Cynodon dactylon in letrozole induced polycystic ovarian syndrome in Wistars albino rats. Anat Cell Biol. 2019 Dec;52(4):511-517.

PCOS is induced by the use of oral drug letrozole for 21 to 28 days or intramuscular injection estradiol valerate. In order to verify PCOS in rodents, estrous cycle is noted from day 1 to 21, as well as changes in ovulation phases determined by amount of cornified cells, nucleated epithelial cell leukocytes in vaginal smear morphology. In the estrous cycle, the animals remain static in the diestrus phase, which is predominantly leukocyte cells [7, 8]. Numerous studies validate the diestrus phase in PCOS, but none have disclosed the estrous cycle processing pattern in rodents. The above research was done to fill this lacuna. Evaluation of vaginal cytology is particularly used to determine the mating period of rodonts and to reduce pseudopregnancy [9]. A regular estrous cycle shows consistent internal change in vaginal epithelial cytology. This is directly related to the phases of vaginal, uterine and ovarian changes in reproductive hormones and their impact on the target organs [10]. Thus, vaginal cytology can reveal the alteration in the

steroid genic condition of the rodent models and this can be used as a resource for detecting PCOS in animal models to protect the mortality of the species.

Cynodon dactylon or Bermuda grass is seen in moderate climate all over the world between south and north latitudes. C. dactylon is a stoloniferous, hardy perennial grass, very much variable with long rapid growing, rooting at nodes, forming a dense tuft on the top of the soil [11]. C. dactylon is widely used for traditional medical practice in India [12]. Crude extract of this plant is used for treatment of cancer [13], obesity, diabetic [14] gastric ulcers [15], etc. There is also evidence for its antihyperlipedemic [12], hepatoprotective [16] antimicrobial [17, 18], and anti-atherosclerotic [19] properties of this plant.

2.1. International Status:

C. dactylon is used by traditional healers for purifying the blood, diarrhea, gonorrhea, conjunctivitis, anuria, biliousness, itches, and stomach ache [11]. The literature survey also reveals that the dried extracts of C. dactylon aerial parts were investigated for CNS activities in rat [5]. Other essential functions of this plant include analgesic and antipyretic, antiulcer, antihypertensive, antihysteric, antipyretic, antibiotic, antikidney stone, antiviral, antipsychotic, antigonorrheal infection as well as hypoglycemic agent [11, 14, 15]. Studies on lab animals have shown that methanolic extract of C. dactylon decreases the level of lipid peroxides. It was also revealed that the methanolic extract of C. dactylon had an antioxidant effect on COLO 320 DM cells, a colon cancer cell line, and the levels of antioxidant enzymes [6].

2.2. National Status:

Menstrual cycle is controlled by luteinizing hormone (LH) and follicle-stimulating hormone of anterior pituitary and regulated by gonadotropin-releasing hormone of hypothalamus. Any disturbance to this regulatory mechanism alters the pulsatile release of these hormones, especially LH; leads to polycystic ovarian syndrome (PCOS). Changes in vaginal cytology are used to interpret the changes in hormonal levels and modifications in estrous cycle. The aim of this study is to compare the pattern of vaginal cytology and body mass among PCOS rats which are treated with metformin and Cynodon dactylon. Twenty-four Wistar rats were selected and divided into four groups: control, induced, treatment, and referral. PCOS was induced in all groups except controls by giving letrozole through oral gavage for 21 days. After inducing PCOS, the referral and treatment group were treated for PCOS with metformin and C.

dactylon respectively for next 21 days. Vaginal smear of all the groups were taken every day from day one and screened for estrous cycle. The body mass of the animals was measured on days 1, 21, and 42. Animals were sacrificed after 24 hours of the last dose and the reproductive organs were dissected out and weighed. Results of the study show the estrous cycle begins to revert after 1-week administration of C. dactylon; while the changes were slower in referral group. There was a rapid decrease in the body mass as well as reproductive organs among the treatment and referral group compared to that of induced and control. Finding of this study suggests that C. dactylon treats PCOS better than metformin.

3. Progress/ achievement so far, if any

a) Reference papers were collected.

b) Literature survey was studied.

c) Experiments were designed and proposal has been sent for ethical clearence

4. Work plan

4.1 Methodology

The study is planned in Sri Lakshmi Narayana Institute of Medical Sciences, Pondicherry Tamil Nadu, after obtaining due institutional, animal ethical clearances. Twenty-four Wistars albino rats were taken and divided into four groups of six animals in each. The groups were follows: control group, induced (PCOS) group, referral group (metformin 100 mg/kg), and treatment group (C. dactylon 500 mg/kg).

Plant material

C. dactylon plant was collected from the campus of Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry. One hundred grams of plant powder was mixed with 1,000 ml of distilled water and heated until boiling. The mixture was filtered and lyophilization was done. Vaginal smear Each animal was taken off the cage, a wet cotton swab was inserted into the vagina of the animal while carefully holding the tail in one hand. The wet cotton swab was gently rotated and removed out of the animal. Using the wet cotton swab,

a smear was created on a clean grease-free microscope slide. The slides were air-dried and stained with methylene blue or crystal violet stain and observed under a binocular microscope to identify different stages of estrous cycle.

Stages of estrous cycle

Proestrous

The proestrous is defined by the existence between cells of small, round, nucleated epithelial cells with resemblance in form. They are also numerous in numbers. The nuclei are basophilic, and the cells are seen in clusters. There are also mostly nucleated and some cornified epithelial cells.

Estrous

The estrous is defined by extensive cornified epithelial cells without nuclei and some well-developed nucleated epithelial cells.

Metaestrous

The metaestrous is defined by the presence of both predominately cornified epithelium with nucleus and without nucleus as well as a few neutrophils (Fig. 3). Mostly cornified epithelial cells, neutrophils, and some nucleated epithelial cells are present.

Diestrous

Diestrous is featured by having a greater number of neutrophils and lesser number of cornified epithelial cells.

Animal study

The selected animals weighed between 125 and 150 g and were in an estrous cycle. All of the animals had free access to food and water. Twenty-four rats were examined in the everyday vaginal cycle. Animals in groups 2 to 4 were administered letrozole with oral feeding needle for 21 days in the first stage (induced). Vaginal smear was examined to confirm development of PCOS. In the second stage (treatment), 22–42 days, the animals in groups 3 and 4 were treated with Bermuda grass extract and metformin respectively. The animals were weighed periodically

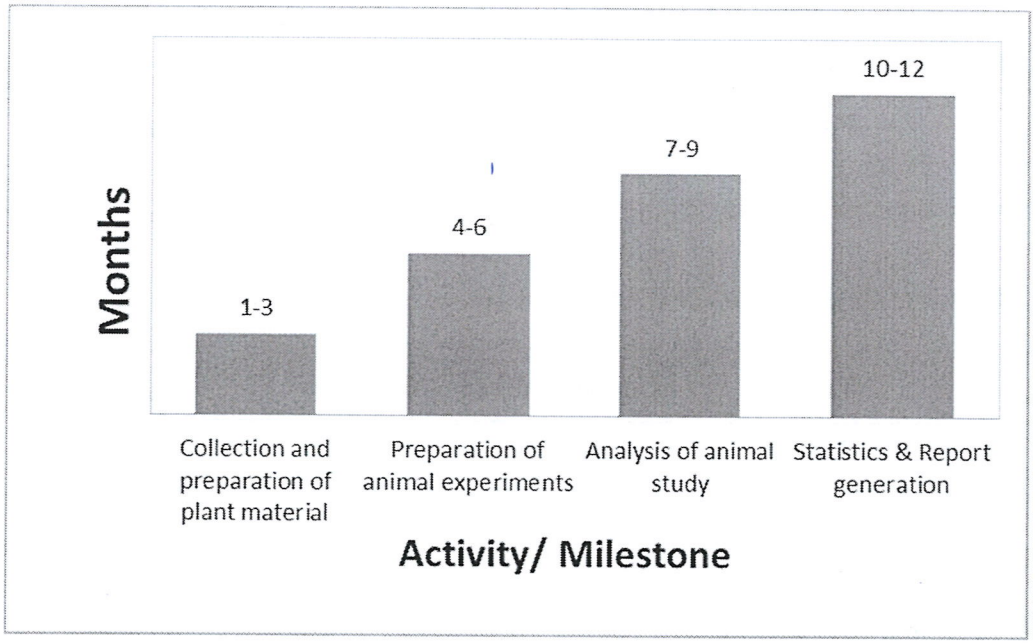
on first day of induction, on the 21st day and on the 42nd day. After 24 hours from the last dose of Bermuda grass extract and metformin the animals were anesthetized, decapitated and dissected.

After 24 hrs of the last dose of the drug and metformin, the animals were anesthetized with overdose as per the standard animal experimental procedure.

The blood collected in a vacutainer tube (serum, plasma, and EDTA) by direct heart puncture. The serum tube was allowed to clot and centrifuged at 3000rpm for 15 minutes and serum was separated and kept stored at-20 degrees. This serum was used for the estimation of the hormonal assay (LH, FSH, Estradiol, and Testosterone) using Enzyme-linked immunosorbent assay(ELISA). Lipid pro□ile parameters (HDL-C, LDL-C, total cholesterol and triglyceride levels) and plasma glucose were analyzed by Merck kit method using an auto-analyzer.

4.2 Time Schedule of activities giving milestones through BAR diagram.(Maximum of 1/2 pages)

S.No	Activity/ milestone	1st Year			
1.	Collection and preparation of plant material	**1-3 month**			
2.	Preparation of animal experiments	-	**4-6month**		
3.	Analysis of animal study	-	-	**7-9 month**	
4.	Statistics & Report generation	-	-	-	**10-12month**



4.3 Expected outcome within the time period of See Money Scheme

Normally the estrous cycle comprises of four phases: proestrous, estrous, metaestrous, and diestrous. A full cycle of the estrous takes 4 to 5 days. Letrozole is an inhibitor of aromatase which induces PCOS in animal models. In most studies, letrozole is used to induce PCOS in rodent models and corresponding changes in the cytological features of vaginal smear, indicating the estrous cycle to be arrested in diestrous phase.

5. Suggested Plan of action stating the name of funding agency where the project will be communicated for financial support within the time period of project.

Nil

6. Bibliography:

Nil

7. List of Projects submitted/implemented by the Investigators (Separate for PI and Co-PI)

7.1 Details of Projects submitted to various funding agencies:

S.No	Title	Cost in Lakhs	Month of Submission	Role as PI/Co-PI	Agency	Status
1	NA	NA	NA	NA	NA	NA

7.2 Details of Projects under implementation

Sl.	Title	Cost in	Duration	Role as PI/ Co-PI	Agency
1	NA	NA	NA	NA	NA

7.3 Details of Projects completed during the last 5 years

Sl.	Title	Cost in Lakhs	Duration	Role as PI/ Co-PI	Agency
1	NA	NA	NA	NA	NA

8. List of publications published by the Investigators, if any:

a) Principal Investigator

S.No	Author names	Title of paper	Name of Journal	Vol (Issue)	Page No.	Year
1	**Anandaramajayan Nallathambi1,** Rajesh Bhargavan2	GC/MS Analysis of Bioactive Compounds in Aqueous Extract of Cynodon Dactylon	Indian Journal of Public Health Research & Development,	10(12)	55-59	2019
2.	Vijisha Phalgunan, **Anandaramajayan Nallathambi**	A Study on Bifurcation of Brachial Artery in South Indian Population(Tamil Nadu and Puducherry)	Indian Journal of Anatomy	7(1)	73-78	2018
3.	N.**Anandaramajayan***, K.C.Mallikarjuna.	Fused Typical Cervical Vertebra – A Case Report	Journal of Current Trends in Clinical Medicine & Laboratory Biochemistry	2(4)	64-66	2015
4.	N.**Anandaramajayan***, B.Rajesh.	Unilateral Renal Agenesis with variations in the vascular pattern of Testis, Supra Renal Gland And Diaphragm -A Case Report	Journal of Current Trends in Clinical Medicine & Laboratory Biochemistry	2(2)	66-72	2014
5.	B. Rajesh*, 2N.**Anandaramajayan**, V.Santhi, K.C. Mallikarjuna,S.I. Tolanur, R. Praveen Kumar	An abnormal radicle of Median Nerve from Musculocutaneous nerve in the Arm	Journal of Current Trends in Clinical Medicine & Laboratory Biochemistry	1(2)	34-36	2013

b). Co-Principal Investigator

S. No	Author names	Title of paper	Name of Journal	Vol (issue)	Page no.	Year
1.	Arivalagan Arunkumar, **Bhargavan Rajesh,** V. Tamilalagan	Variations among foramen transversarium in cervical vertebrae and its clinical significance	Indian Journal of Anatomy	7(2)	144-148	2018
2.	Mary Hydrina D'Silva1, Rijied Thompson Swer2, J. Anbalagan3, **Bhargavan Rajesh 4**	Effect of Radiofrequency Radiation Emitted from 2G and 3G Cell Phone on Developing Liver of Chick Embryo – A Comparative Study	Journal of Clinical and Diagnostic Research	11(7)	AC05 - AC09	2017
3.	M. Senthil Murugan,[1,*] R. Sudha,[1] and **Rajesh Bhargavan[2]**	Clinical Significance of an Unusual Variation Anomalous additional belly of the sternothyroid muscle	Sultan Qaboos University Med J,	16(4)	e491–494,	2016
4.	Mary Hydrina D'Silva,1 Rijied Thompson Swer,1 J. Anbalagan,1 and **Rajesh Bhargavan2**	Effect of Ultrahigh Frequency Radiation Emitted from 2G Cell Phone on Developing Lens of Chick Embryo: A Histological Study	Advances in Anatomy	10(2)	1-9	2014
5.	Vasudev Anand Rao, Subashini Kaliaperumal, Thanikachalam	Goldenhar's sequence with	Indian Journal Of	53(4)	267-268	2005

	Subramanyan, Kotapalli Rachandra Rao, **Rajesh Bhargavan**	associated juvenile Glaucoma in turner's syndrome	Ophthalmology			

9. Budget

SI. No	Head	Amount (Rs.)
1	SLIDES CONSUMABLES	50,000
2	REAGENTS	25,000
3	Travel support for the purpose of research work.	10,000
4	Contingency	10,000
5	Others	5,000
	Total	**1,00,000/-**

*In case of any joint proposal for purchasing a same equipment, each of the associated PLs is also required to give separate budget(without any clubbing) to avoid any ambiguity, if all the associated projects are not awarded by committee.

10. Name of at least two subject experts from the Institute and one from the outside Institute with their contact details:

1. **Dr. T K Balaji,** Professor in Anatomy, Chettinad Medical College Hospital and Research Centre, Chennai **Mobile No**: 9710905221 **E-mail id:** balajitk@gmail.com	2. **Dr. Senthil Kumar** Associate Professor in Anatomy, AIIMS, Nagpur **Mobile No**: 8524863767 **E-mail id:** senkumar@yahoo.co.in

CERTIFICATE FROM THE INVESTIGATOR

Project Title: Regulation of estrous cycle by Cynodon dactylon in letrozole induced polycystic ovarian syndrome in Wistars albino rats

It is certified that

1. I do hereby agree to submit a complete proposal for financial support to the external funding agency within the time period of SMS-2018.
2. I undertake that spare time on equipment procured in the project will be made available to other users.
3. I agree to submit a certificate from Institutional Biosafety Committee, if the project involves the utilization of genetically engineered organisms. I also declare that while conducting experiments, the Biosafety Guidelines of Department of Biotechnology, Department of Health Research, GOI would be followed in to.
4. I agree to submit ethical clearance certificate from the concerned ethical committee, if the project involved field trails/experiments/exchange of specimens, human & animal materials etc.
5. I agree to abide by the terms and conditions of SMS-2018, BIHER, and Chennai.

Name and signature of
Principal Investigator

Name and signature of
Co-Principal Investigator

Date: 09.01.2019
Place: Pondicherry

Forwarded by Head of the Department

Signature of the Head

DEAN
SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSUDU, AGARAM VILLAGE
KOODAPAKKAM POST
PUDUCHERRY - 605 502

PROJECT EVALUATION FORMAT

Recommendation sheet

Name of the Principal Investigator	N.Anandaramajayan
Name of the Co-Principal Investigator	B.Rajesh
Name of the Department	Anatomy
Title of project	Regulation of estrous cycle by Cynodon dactylon in letrozole induced polycystic ovarian syndrome in Wistars albino rats
Recommendation of the evaluation committee (Recommended/Revision/Not Recommended)	Recommended
Financial allocation recommended	Rs. 1,00,000/-

SI. No	Head	Amount (Rs.)
1	SLIDES CONSUMABLES	50,000
2	REAGENTS	25,000
3	Travel support for the purpose of research work.	10,000
4	Contingency	10,000
5	Others	5,000
	Total	**1,00,000/-**

Name and Signature of the Research Advisory Committee members with date.

Internal Quality Assurance Cell ★ IQAC ★ Pondicherry-01 ★ SLIMS

(Dr. G. Jayalakshmi).