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)
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Chennai - 600 073. Tamil Nadu.

Ref. No.SMS-2018-O-07

Date: 06.02.2020

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: Biochemical and Hormonal Profile of Letrozole Induced PolycysticOvarian Syndrome in Wistars Albino Rats treated with Cynodon dactylon

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

Approved on: 03.02.2020

Payment details:

Voucher No.62 **Dated: 12.02.2020**

With Regards

Dean-Research

Bharath University

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

CASH / PAYMENT VOUCHER

Date 12/02/2020

V.No. 62

Debit _____ Amount

Rs. 1,00,000/-

PAID TO Dr. Anandavarna Jayan

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	: 01-12-2010

2. **Details of Co - 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 polycystic ovarian syndrome is a heterogeneous disorder with an incident of 7% and it increases every year by 0.56% to1.14% worldwide and in India, it is about 2.2 to 26% (Nidhi et al., 2011; Christensen et al., 2013). The syndrome is associated with an increase in androgen, hyperinsulinemia, secretion of a high level of luteinizing hormone, weight gain, anovulation, cyst formation, amenorrhea leading to hirsutism and ovarian cancer (Mohan and Vignesh, 2007; Pasquali et al., 2011). LH and FSH are the hormones that control the menstrual cycle. This is regulated by the pulse frequency of hypothalamic GnRH secretion. An abnormal change in this pulse frequency leads to polycystic ovarian syndrome (Wildt et al., 1981; Reame et al., 1984; Hayes, 1998). Metformin is the present treatment for PCOS, which is an insulin sensitizer with more side effects like nausea, vomiting, mood change, etc. (Mathur et al., 2008; Soyman et al., 2017). In order to prevent the side effect and to ind a better cure, Cynodon.dactylon. the present drug was selected. Thakare et al., 2011), and anticancer (Venkateswarlu et al., 2015). The present study is to find alternative natural medicine to treat PCOS without the side effect of the modern drug and a better cure.

Cynodon dactylon is commonly called Arugampul, Bermuda grass, or dog's tooth grass. The creeping grass is mainly seen in 45 degrees north and 45 degrees south latitude (Asthana et al., 2012; Rita et al., 2012). Arugampul is used for offerings in temple and various medicinal uses like diabetes (Mahesh and Brahatheeswaran, 2007; Madhankumar, 2016), antioxidants (Eskandary et al., 2017; Pawaskar and Sasangan, 2017) antidiarrheal (Rahman et al., 2015) antihyper lipidemic (Kaup et al., 2011), antimicrobial and antifungal (Rao et al., 2011; Bagewadi et al., 2014), anti-inflammatory and wound healing (Garg and Paliwal, 2011).

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

Ashish, Anjali, Praveen K Dixit, & Nagarajan K. (2021). Pharmacological and Biochemical evaluation of anti-arthritic activity of Justicia gendarussa extract in FCA induced arthritis in Wistar rats. International Journal of Research in Pharmaceutical Sciences, 12(3), 1699-1708.

Inflammation is a response of immune system that protects the host against the foreign substance. The major four vital signs of acute inflammation are as follows, Calor (heat), Rubor (redness), tumor (swelling) and dolor (pain), leading to Functiolaesa loss (or impairment) of

function (Mittal, Siddiqui, Tran, Reddy, & Malik, 2014). Inflammatory process which is successful or controlled in manner is needed that leads to the removal of dangerous stimuli and also help in the restoration of normal physiology (cellular or tissue physiology) (Tasneem, Liu, Li, Choudhary, & Wang, 2019).

The major targets of rheumatoid arthritis is synovial joints which leads to the destruction of the synovial joints and their membrane causes joint disability, arthralgia, swelling, redness and also limit the joint movement (Guo et al., 2018). Arthritis is a common term with a joint paint as a primary feature which includes osteoarthritis (OA), rheumatoid arthritis (RA) and psoriatic arthritis (Li et al., 2016). In traditional medicine system the plant aerial part of Justicia gendarussa (f. Acanthaceae) is very well known for its various therapeutic activities (Ramees et al., 2019). Leaves contain, alkaloids, flavonoids, saponin, steroids, O-distributed aromatic amine i.e., 2-(2'-amino-benzylamino) benzyl alcohol and their respective O-methyl ethers, stigmasterol, lupeol, 16-hydroxylupeol, 28β-sitosterol (Ashish, Anjali, K, K, & Jagannath, 2020). The plant shows the various therapeutic activity like, anti-arthritic (Paval et al., 2009; Paval et al., 2009), anti-inflammatory (Kavitha, Viji, Kripa, & Helen, 2011), anti-bacterial (Kumar, Sabu, Sindhu, Rauf, & Helen, 2018), dyspepsia (Kumar et al., 2012), cytotoxic (Ayob, Bohari, Samad, & Jamil, 2014), anti-angiogenic (Mangai, 2018), anti-fungal (Ayob, Samad, & Bohari, 2013), Cough (Patel & Zaveri, 2012), hepatoprotective (Krishna, Mruthunjay, & Patel, 2010; Phukan, Kakoti, Verma, & Kumar, 2014), anti-anxiety activity (Jothimaniv, Subramania, Kumar, & Kameshwara, 2013), anti-depressant effect (Mythili & Jothimanivannan, 2017), sedative and hypnotic activity (Jothimaniv et al., 2013), anti-diabetic (Islam, Sayeed, Billah, Nasiruddin, & Alam, 2015).Normally the estrous cycle comprises of four phases: proestrous, estrous, metaestrous, and diestrous. A full cycle of the estrous takes 4 to 5 days [8]. 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 [21,22]. In the existing research, vaginal cytology was used as an indicator of inversion of the estrous cycle in contrast between drug group and metformin group. Comparing the mean body weight, there was an increase in all groups except the control group on the 21st day. However, after day 21, there was a fast reduction in body weight in treatment and referral group. The weight of the right and left ovaries and the weight of the uterus was found to be increased in the induced group and decreased in treatment and referral groups. These changes have also been observed in other studies

[23,24,25,26,27]. Nevertheless, the uterine mass was reduced in the treatment group compared to that of the referral group in our study. This is mainly due to the antihyperlipedemic [12] and decrease in insulin resistance [14] activity of the C. dactylon.

The restoration of esterous cycles occurred a day earlier in PCOS animal models treated with water-soluble C. dactylon (Bermuda grass) extract compared to that of metformin-treated group. 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.

2.1. International Status:

Nil

2.2. National Status:

Research has been done on the anti- inflammatory work of various extract Justicia gendarussa by using the in-vitro and in-vivo model sand the extract JGLE, JGLC, and JGSE is used to evaluate for their anti-inflammatory activity. In the anti-inflammatory (in-vitro) study there are2 models (Inhibition of protein denaturation and Human red blood cell membrane stabilization method) are used and the effect of these extracts for these in vitro-study were measured or evaluated at concentration (50, 100, 200, 400, 800, 1000, 2000g/ml) and (50, 100, 200 400, 800, 1600g/ml)respectively. Effect of the test extract increases withthe concentration and the extract JGLE to be foundmost effective at the concentration of 2000g/mlin both the models and the results of the extractswas compare with the reference drug, Diclofenac sodium, at the same concentration. Carrageenan paw edema model is used as in-vivo method forthe evaluation the anti-inflammatory potential of the test extract JGLE, JGLC, and JGSE which is obtained from the aerial part of Justicia gendarussa plant. The plant extract (JGLE, JGLC and JGSE) concentration 50mg/kg/b.w.p.o is used and the result is compare with the reference compound Diclofenac sodium at 20mg/kg/b.w.p.o. The extract JGLE has been shown more paw edema inhibition than the other extracts. The anti-arthritis ability of the plant Justicia gendarussa extract as JGLE, JGLCand JGSE was evaluated using the arthritis model induced by FCA in Wistar rats.

3. Progress/ achievement so far, if any

a) Reference papers were collected.

b) Experimental design planned.

c) Proposal work has been started in collection of drug and ethical clearence

4. Work plan

4.1 Methodology

The study was designed in Sri Lakshmi Narayana Institute of Medical Sciences Pondicherry Tamil Nadu, after obtaining the proper clearance from institutional animal ethical committee. 36 Wistars albino rat was taken and divided into six groups with each group six animals. Control group, Induced (PCOS) group Letrozole 1mg/kg with 0.5% . Carboxymethyl cellulose, Referral (Metformin) group (100mg/kg with 0.5%CMC, Treatment group (C. dactylon) 500mg/kg,1,000 mg/kg,1,500 mg/kg with 0.5%CMC.

Plant Material

Cynodon dactylon plant was collected from the campus of Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry. The plant was cleaned with distilled water and air-dried in the room temperature and grinded to powder in the grinding machine. The 100 gm of plant powder was mixed with 1000 ml of distilled water and heated till boiling temperature. The mixture was filtered using Whatman's no: 1 filter paper. Then lyopilized to powder form.

Experimental design

The animals were selected based upon the weight 125-150 gm and oestrus cycle. The animals were kept in a polypropylene cage with free access to food and water. The animals were examined for the normal vaginal cycle. In 1st phase except for the control animals, all the animals are induced for PCOS by giving Letrozole with an oral gavage for 21 days and the vaginal smear was examined to confirm PCOS. In the 2nd phase (Drug) 22-42 days, the animals were treated with the drug and Metformin. The animalswere divided into five groups as Induced group, treatment group with 500 mg, 1,000 mg, 1,500mg and Referral group.

Bio-Chemical Analysis

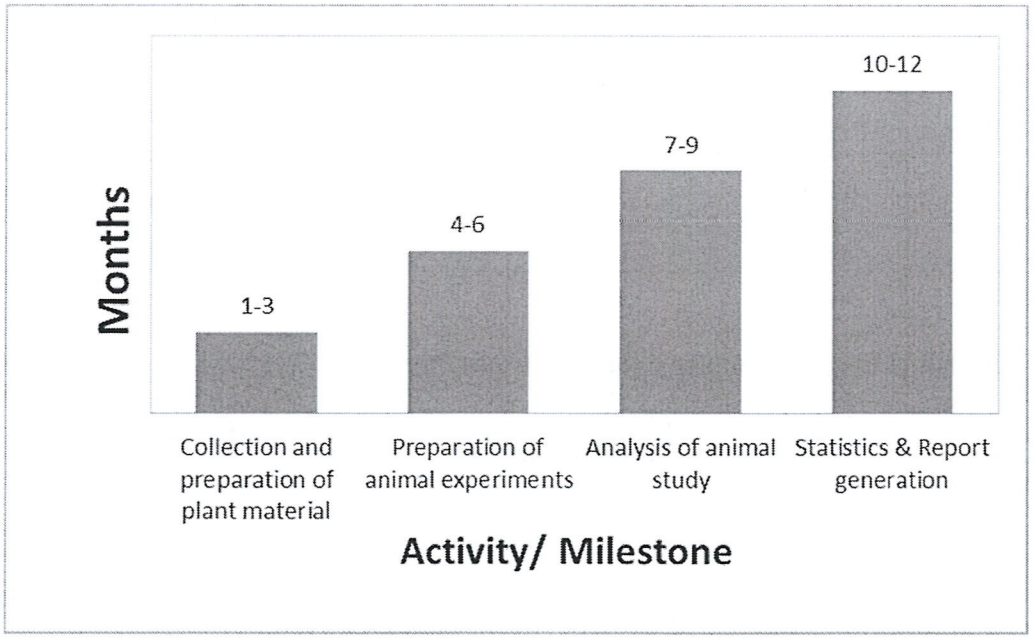
After 24 hrs of the last dose of the drug and metformin, the animals were anesthetized with overdose as per the standard animal experimental procedure. The blood was collected in a vacutainer tube (serum, plasma, and EDTA) by direct heart puncture. The serum tube was allowed to clot and centrifuged at 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

Statistical Analysis

Statistical analysis was done using one-way analysis of variance(ANOVA) and Tukey's post hoc test for multiple comparisons to test the significant ($P < 0.05$) between groups using SPSS program v14.00.The Result values are shown with Mean_S.D.

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

The medicinal effects of C. dactylon in Letrozole induced Polycystic Ovarian Syndrome in rat models. Our study can prove that C. dactylon is effective in changing the lipid profile of PCOS compared to Metformin. There were also significant changes in hormonal and glucose profile compared to Metformin. It reverts the animal to better breeding conditions compared to that of Metformin and Control.

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 Nallathambi1,2,** Rajesh Bhargavan1,2	Regulation of estrous cycle by Cynodon dactylon in letrozole induced polycystic ovarian syndrome in Wistars albino rats	Anat Cell Biol	52	511-517	2019
2.	**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)	54-59	2019
3.	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
4.	N.**Anandaramajayan** *, K.C.Mallikarjuna.	Fused Typical Cervical Vertebra – A Case Report	Journal of Current Trends in Clinical Medicine &	2(4)	64-66	2015

			Laboratory Biochemistry			
5.	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
6.	B. Rajesh*, 2N.**Anandaramajaya n**, 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

9. Budget

SI. No	Head	Amount (Rs.)
1	ELISA reader	50,000
2	Reagent kits	30,000
3	Travel	10,000
4	Contingency	5,000
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: Biochemical and Hormonal Profile of Letrozole Induced Polycystic Ovarian Syndrome in Wistars Albino Rats treated with Cynodon dactylon

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: 10.01.2020
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	Dr. B. Rajesh
Name of the Department	Anatomy
Title of project	Biochemical and Hormonal Profile of Letrozole Induced Polycystic Ovarian Syndrome in Wistars Albino Rats treated with Cynodon dactylon
Recommendation of the evaluation committee (Recommended/Revision/Not Recommended)	RECOMMENDED
Financial allocation recommended	Rs. 1,00,000/-

SI. No	Head	Amount (Rs.)
1	ELISA reader	50,000
2	Reagent kits	30,000
3	Travel	10,000
4	Contingency	5,000
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-02 SLIMS

(Dr. K. BALAGURUNATHAN)