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Expression of IGF-1R, SIRT1, and LEPTIN in the therapeutic effect of *Cynodon.dactylon* in Letrozole induced PCOS rats

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ABSTRACT

Polycystic ovarian syndrome is a female reproductive disorder with a prevalence rate of 2.2% to 26% in the world and Indian adolescents at a rate of 9.13%. It is a heterogeneous endocrine syndrome related to increase in the secretion of hyperandrogenemia with increasing risk of hyperinsulinism, type 2 diabetes mellitus, Hirsutism dyslipidemia, cardiovascular diseases and finally leading to an endometrial tumor. *Cynodon dactylon* or Bermuda grass is seen all over the world. *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. *C.dactylon* plant was collected and 100gm of plant powder was mixed with 1,000ml of distilled water and heated till boiling temperature. The mixture was filtered and lyophilized. All the animals were induced for PCOS by giving Letrozole with an oral feeding needle for 21 days and the vaginal smear was examined to confirm PCOS. From 22-42 days, the animals were treated with the drug and Metformin. The animals were divided with four groups as Control, Induced group, treatment group with 500 mg/kg (*Cynodon dactylon*), and Referral group 100mg/kg (Metformin). After 24hr of the last dose, the animals were sacrificed. Blood and tissue were collected for analysis. IGF-1R. LEPTIN is a gene which mainly acts on the hypothalamus in regulating the gonadotropin release hormone and gonadotropin secretion it regulates the energy level and adipose tissue storage in the body. SIRT1 is a nicotinamide adenosine dinucleotide which increases the insulin sensitivity and decreases insulin resistance by modifying the insulin signal transduction pathway.

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INTRODUCTION

The polycystic ovarian syndrome is a female reproductive disorder with a prevalence rate of 2.2% to 26% in the world and Indian adolescents at a rate of 9.13% (Nidhi *et al.*, 2011). A regular menstrual cycle is defined as characterized by an inter-menstrual interval of not that less than 21 and not more than 35 days with no more than four-day variation from cycle to cycle (Franks, 1989). The polycystic ovarian syndrome is a heterogeneous endocrine syndrome related to increase in the secretion of hyperandrogenemia with an increasing risk of hyperin-

sulinism, type 2 diabetes mellitus, Hirsutism dyslipidemia, cardiovascular diseases and finally leading to endometrial tumor (Azziz *et al.*, 2004).

Proper periodic systemic growth and metabolic function are mainly done by the endocrine organs present in various parts of the body, mainly contributed by the hypothalamus and pituitary. These endocrine organs secrete hormones which are made up of steroids and amino acids. These hormones act on target organs and regulate the secretion by positive feedback, negative feedback and short loop feedback mechanism (Hiller-Sturmhöfel, 1998). The endocrine hormones are regulated by various factors like environment, photoperiod, ambient temperature, relative humidity availability of food, etc. (Kawamoto, 2003). In recent study substances like Bisphonal A are seen in more amount in human blood which extend the length of ester cycle and alter the morphological and functional in genital system in female and which lead to PCOS (Zhou *et al.*, 2008). Apart from this, industrials workers are exposed to endocrine disruptors like, Chlorobenzenes, Parabens, Phthalate, Benzo (A)pyrene, PCB, Chlordane, Butyl tin compounds which disturb the orderly endocrine mechanism and alter the molecular characters to the endocrine hormone (Tsunehisa, 2003; Kandaraki *et al.*, 2011).

Cynodon dactylon or Bermuda grass is seen in a 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.

C.dactylon is widely used for traditional medical practice in India (Kaup *et al.*, 2011). The crude extract of this plant is used for the treatment of cancer (Kanimozhi, 2013), obesity, diabetic (Karthik and Kumar, 2011) gastric ulcers (Ramesh, 2013), etc. There is also evidence for its Antihyperlipidemic (Kaup *et al.*, 2011), Hepatoprotective (Devi *et al.*, 2017). Antimicrobial (Pandey *et al.*, 2016; Sharma, 2016) and Anti-atherosclerotic (Pashaie *et al.*, 2017) properties of this plant.

MATERIALS AND METHODS

The study was designed in Sri Lakshmi Narayana Institute of Medical Sciences Pondicherry. It was executed in JKK Munirajah Medical Research Foundations college of Pharmacy, Tamil Nadu, after obtaining the proper clearance from institutional animal ethical clearance. 24 Wistars albino rat was taken and divided as a Control group, Induced (PCOS) group, Referral group (Metformin group), Treatment group (*Cynodon dactylon*) 500mg/kg each 6 animals.

C.dactylon plant was collected from the campus of Sri Lakshmi Narayana Institute of Medical Sciences Puducherry. The 100gm of plant powder was mixed with 1,000ml of distilled water and heated till boiling temperature. The mixture was filtered and lyophilization.

All the animals were induced for PCOS by giving Letrozole with oral gavage for 21 days and the vaginal smear was examined to confirm PCOS. From 22-42 days, the animals were treated with the drug and Metformin. The animals were divided with four groups as Control, Induced group, treatment group with 500 mg/kg (*Cynodon dactylon*), and Referral group 100mg/kg (Metformin).

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 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 degree. The ovarian tissue was dissected and removed from the animals for analysis of gene expression.

Determination of enzymatic antioxidants

Lipid peroxidation activity was determined by following the method of R. M. A. Hammouda, (Rahman.M.A.Hammouda and M.M.Khalil, 1995). The superoxide dismutase activity was determined by using Van Rossun (Rossum and Plas, 1997). Catalase activity is done by the method outlined by Luck (Luck, 1965). (RNA isolation and quantitative RT-PCR**)** Total RNA was extracted from cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol.

Gene expression

Reverse transcription was performed using Prime Script™ 1st strand DNA Synthesis Kit (TAKARA BIO INC) following the manufacturer's instructions.

Primers used

IGF-1R FP: 5' TCTAAGGCCAGAGGTGGAGAATA 3'

IGF-1R RP: 5' TACCATGCAGTTCCGAGCAG 3'

Leptin FP: 5' GACATTTCACACACGCAGTC 3'

Leptin RP: 5' GAGGAGGTCTCGCAGGTT 3'

SIRT1 FP: 5' GACATTTCACACACGCAGTC 3'

SIRT1 RP: 5' TGGTTCATTTATCAGAGTTGCC 3'

Statistical analysis

Statistical differences between Control, Induced, Treatment and Referral groups were applied using

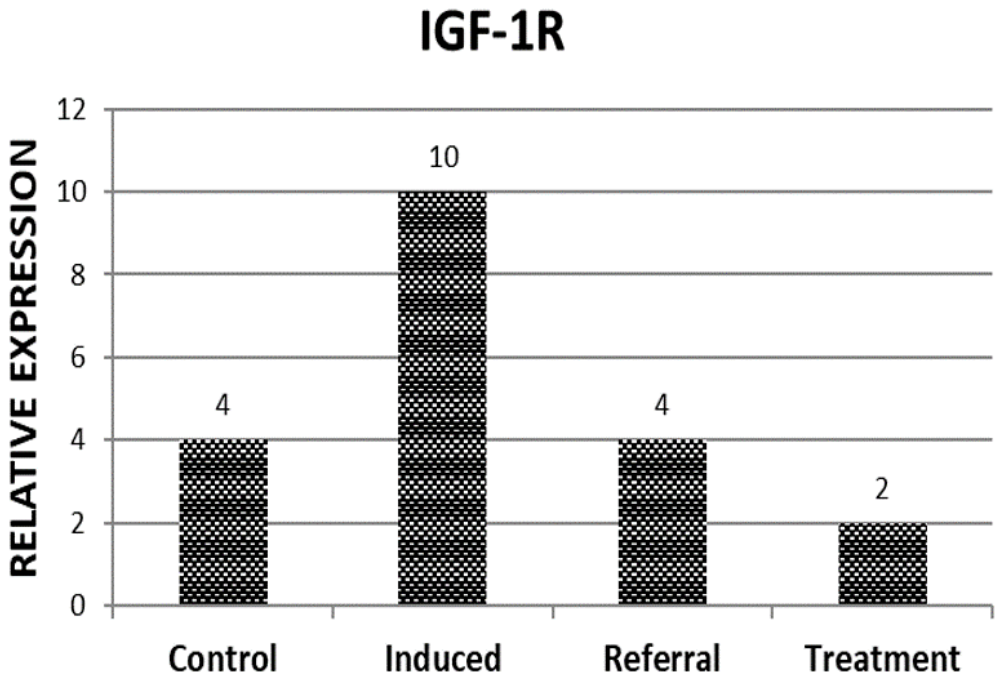

Figure 1: Expression of IG-1R of Control, Induced, Treatment, and Referral group. The Treatment group shows a significant change over the induced and Referral group

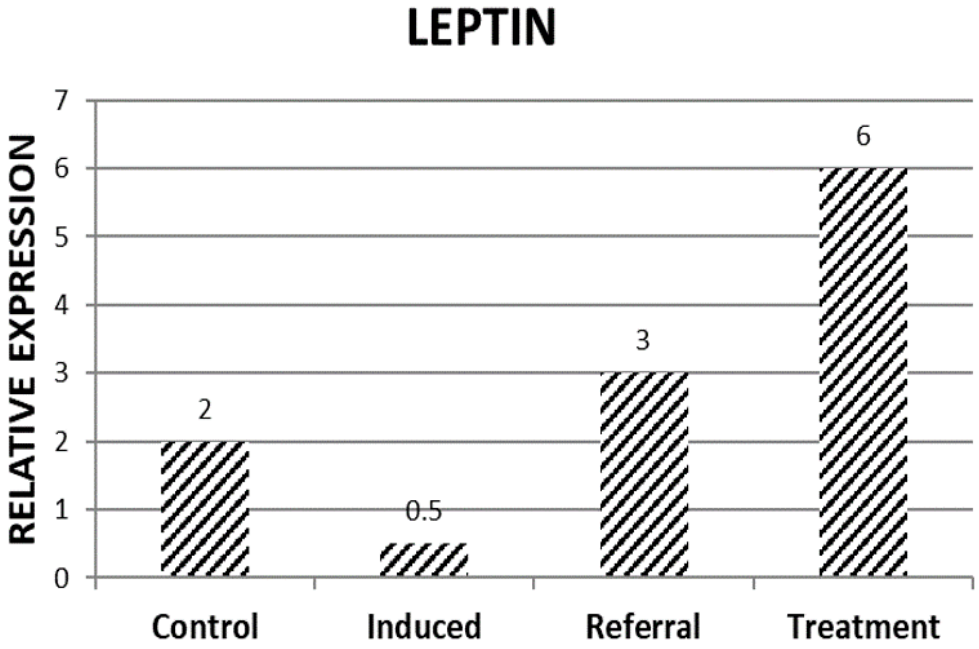

Figure 2: Expression of LEPTIN in Control, Induced, Treatment, and Referral group. The Referralgroup shows a significant change over the induced and Treatment group

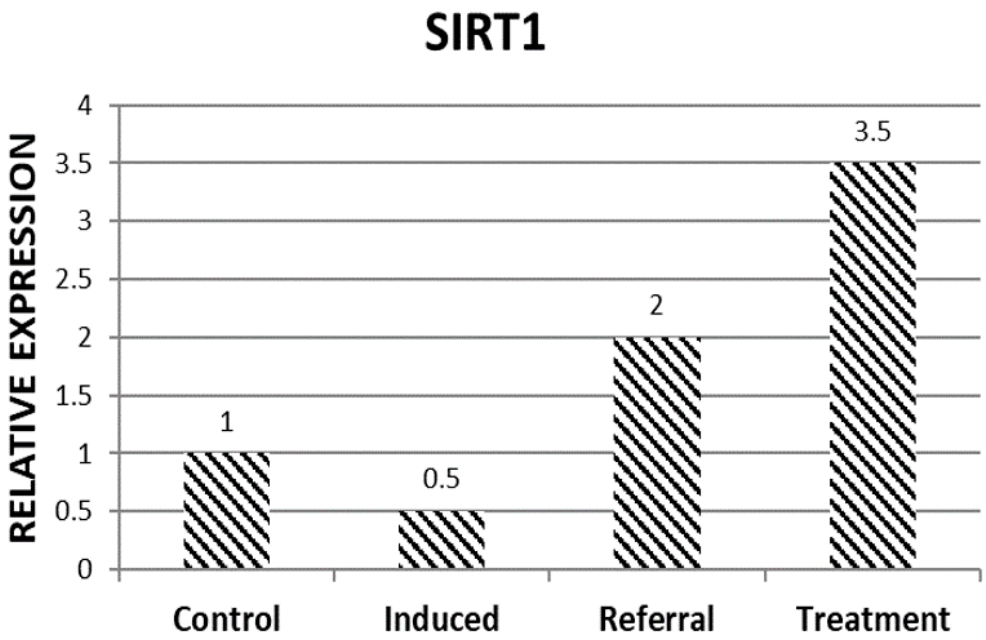

Figure 3: Expression of SIRT in Control, Induced, Treatment, and Referral group. The Treatment group shows a significant change over the induced and Referral group

Graph Pad Prism software version 8 (Graph Pad Software). The results were expressed as mean ± SEM. Results were analyzed using one-way ANOVA, and data were considered to be statistically significant at P <0.05.

RESULTS AND DISCUSSION

Lipidperoxidase Significant changes are observed in control and induced group*P<0.05 and comparing with treatment group*P<0.05. Superoxide Table 1.

Dismutase

There was a decrease in the values of the induced and control group and an increase in the value of the treatment group and it was significant.

Catalase

we are not able to observe any changes in the catalase activity.

Expression of IGF-1R, Leptin & SIRT1

The expression of IGF-1R has increased in the Induced group compared to Control, and the expression of IGF-1R has tremendously decreased in Treatment (P<0.05) of ovarian theca cells and to the level of control in Referral Figure 1.

Leptin expression was decreased in the Induced group of ovarian theca cells compared to Control, and the expression Leptin has significantly increased in Treatment and the level of control and Referral it was similar Figure 2.

The expression of SIRT1 has decreased in the Induced group compared to Control, and the expression of SIRT1 has increased in Treatment (P<0.05) of ovarian theca cells and Referral Figure 3.

The ovarian cycle is controlled by the hypothalamus – anterior lobe of the pituitary-gonadalal axis. This leads to the development of the Graafian follicls (Lopez-Diaz and Bosu, 1992). Letrozole is an aromatase inhibitor which disturbers the normal estrus cycle in the female rats to develop a well-defined PCOS. At present metformin is used for the treatment of PCOS, nevertheless with the side effects on long term systems (Karateke *et al.*, 2018). The study has already shown *C.dactylon* can improve the reproductive activity in normal animals (Nayanatara, 2012). The antioxidant effect of *C.dactylon* has been proved by phytochemical analysis (Pawaskar and Sasangan, 2017). In our observation, significant changes were seen in the gene regulating estrus cycle in between control and induced group and induced and treatment group. IGF-1R. LEPTIN is a gene which mainly acts on the hypothalamus in regulating the gonadotropin release hormone and gonadotropin secretion it regulates the energy level and adipose tissue storage in the body. Expression of this gene may act on mature Graafian follicles and activate 17-α hydroxylase enzyme, which regulates the ovarian cycle (Mani *et al.*, 2011; Rizk and Sharif, 2015; Correia *et al.*, 2017). In our study, we observe IGF-1R is expressed ten folds in the Induced group and it was found to be decreased to 2 folds in Treatment group P<0.05. Identical expression was seen

Table 1: The Lipid peroxidase test, Superoxide Dismutase, of Control, Induced,Treatment, and Referral group

Parameters	Control	Induced	Treatment	Referral
Lipidperoxidase (MDA)nm/ml	16.03±5.88	50.64±6.75#	32.05±4.84*	37.18±5.84
Superoxide Dismutase U/ mg protein	52.18±0.345	46.6±0.415#	47.55±0.125*	50.88±0.115
Catalase μmol / mg protein	0.05±0.003	0.03±0.004	0.05±0.003	0.04±0.004

in Control and Referral group. Leptin expression was found to be half fold in the induced group and six folds in Treatment group P<0.05. In the Referral group, it was three folds and in Control, it is two folds. This is mainly due to the antilipidemic activity of *C.dactylon* (Kaup *et al.*, 2011). SIRT1 is a nicotinamide adenosine dinucleotide which increases the insulin sensitivity and decreases insulin resistance by modifying the insulin signal transduction pathway. Decreasing in the SIRT1 expression leads to insulin-related disorders (Tao *et al.*, 2015). SIRT1 expression was found 0.5 in the Induced group and three and a half fold in Treatment P<0.05 and Referral group was two folds and it was lesser in Control as single folds. This is mainly due to the antidiabetic activity of C.dactylon (Karthik and Kumar, 2011).

CONCLUSIONS

Our study is the first study to conclude that *Cynodon.dactylon* can treat polycystic ovarian syndrome. The Expression of LEPTIN, IGF-1R, SIRT1 gene shows clearly, the reversal of the animal to normal condition is mainly due to the antihyperlipedemic and antidiabetic activity of the plant.

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