Available online at **www.ijmrhs.com**

ISSN No: 2319-5886 **International Journal of Medical Research and Health Sciences, 2022, 11(8): 1-12**

Effects of Saw-Palmetto (*Serenoa repens*) in Letrazole Induced Poly Cystic Ovarian Syndrome in Female Albino Wistar Rats

V Sharath Kumar*, Shivalinge Gowda KP

Department of Pharmacology, PES University, Karnataka, India

**Corresponding author email: vsharathsharu@gmail.com*

Received: 24-Jun-2020, Manuscript No. IJMRHS-20-14068; **Editor assigned:** 29-Jun-2020, PreQC No. IJMRHS-20-14068 (PQ); **Reviewed:** 13-Jul-2020, QC No. IJMRHS-20-14068 (Q); **Revised:** 09-Sep-2022, QI No. IJMRHS-20-14068; Manuscript No. IJMRHS-20-14068 (R); **Published:** 07-Oct-2022

ABSTRACT

*To investigate benefical effects of saw palmetto (*Serenoa repenes*) in letrazole induced PCOS in female albino rats. Female albino wistar rats were divided in to 5 groups (n=6), Group-I considered as normal control. PCOS was induced in Group-II, Group-III, Group-IV and Group-V rats by oral administration of letrazole (1 mg/kg b.w.p.o) daily for 21 days. After Induction of PCOS Group-III, Group-IV were treated with treatment drug with saw P (medium dose 160 mg/kg and high dose 320 mg/kg) and Group-V is treated with standard drug Clomiphene Citrate (CC) respectively for 22nd to 46th day (25 days) of the study period. Physical parametrs (body weight, ovary and uterus weight), metablolic parameters (OGTT), Heart Rate (HR), Serum parameters, oxidative stress and histopathology were performed. Female albino wistar rats treated with Saw P (Group-III), Saw P (Group-IV) have shown significant decreased in body and ovarian weight, increased uterine weight, decreased total cholesterol, TG, VLDL-C and LDL-C, increased in HDL-C when results were compared with letrozole induced PCOS rats. Group-III, Group-IV and Group-IV rats also showed significant increased in SOD, catalase and GSH when compared with PCOS induced rats. Histological results showed that saw palmetto treatment in PCOS rats resulted in well-developed antral follicles; normal granulosa cell layer and formation of well develop oocyte in rat ovary. From the results of the study it is concluded that saw palmetto (320 mg/kg) treatment significantly altered the letrozole induced PCOS symptoms may due to its antiandrogenic property so, it may be beneficial in PCOS.*

Keywords: Letrozole, Saw palmetto, Serum lipid profile, Clomiphene citrate

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is one of the most common reproductive endocrinological disorders with a broad spectrum of clinical manifestations affecting about 6.8% of women of reproductive years. A polycystic ovary is an abnormally large numbers of developing egg seen to the ovarian periphery, looking like a “String of pearls”. PCOS was previously called Stein-Leventhal syndrome after the physicians who first characterized it in 1930’s. It is associated with oligomenorrhoea, anovulation (causing endocrine disorder such as thyroid disease, adrenal disease and pituitary tumors), infertility, hirsutism and obesity in young women having bilaterally enlarged cystic ovaries. It is a condition in which women have high levels of male hormones (androgen) and anovulation are known to interact with insulin resistance in the pathophysiology of PCOS. Hyperinsulinaemia appears to interfere with ovarian steroidogenic defects as well as anovulatory mechanisms. Child and adolescent overweight and obesity were associated with significantly increased risk of later polycystic ovary syndrome symptoms. Dyslipidemia has a prevalence of 70% in women with PCOS and has been linked with hyperandrogenism and IR. Hyperandrogenemia and IR affect lipid profile in PCOS *via* mechanisms relating to cholesterol metabolism, uptake and efflux from peripheral cells. They also increase immune cells activation to release pro-inflammatory cytokines and enhance excessive production [1].

Causes of PCOS

Genetic predisposition, strong stimulation in adrenals in childhood, raised insulin levels, contraceptive pills, hormonal imbalance, stress PCOS is more common in women with epilepsy (13%-25%) than in general female population (4%-6%), through several mechanisms. The modified function of Hypothalamic-Pituitary Axis (HPA), including production of Luteinizing Hormone (LH), follicle-stimulating hormone and consequently on the Hypothalamic-Pituitary Gonadal (HPG) axis and reproductive function. Studies that have specifically addressed the association between psychotropic medications, on the one hand and menstrual abnormalities, Polycystic Ovary Syndrome (PCOS) and overall reproductive endocrine function in women with BD. Hence this study was designed to develop the PCOS animal model is an analogous human PCOS phenotype by using antiepileptic agents. Saw Palmetto Extract (SPE), an extract from the ripe berries of the American dwarf palm, has been widely used as a therapeutic remedy for urinary dysfunction due to Benign Prostatic Hyperplasia (BPH) in Europe. Numerous mechanisms of action have been proposed for SPE, including the inhibition of 5α-reductase. Today, α1-adrenoceptor antagonists and muscarinic cholinoceptor antagonists are commonly used in the treatment of men with voiding symptoms secondary to BPH. The improvement of voiding symptoms in patients taking SPE may arise from its binding to pharmacologically relevant receptors in the lower urinary tract, such as α1-adrenoceptors, muscarinic cholinoceptors, 1,4-dihyropyridine receptors and vanilloid receptors [2].

MATERIALS AND METHODS

Thirty albino wistar female rats were used in this study. Animals were approved from IAEC and animals collected. Animals were maintained under controlled condition of temperature (23°C ± 2°C), humidity (50% ± 5%) and 12-hour light and dark cycles. Animals were approved from IAEC. IAEC approval was obtained from the Institutional Animal Ethics Committee (PESCP/IAEC/86/2019 dated 20-6-2019) of PES College of Pharmacy, Bengaluru-560050 and the study was conducted in the PCSEA approved animal house (CPCSEA Reg No-600/PO/Ere/S/02/CPCSEA) of PES College of Pharmacy, Bengaluru, according to the guidelines of CPCSEA, New Delhi and animals collected [3].

Experimental design

The animals were randomly assigned into five groups of which n=6 animals per group; control, PCOS, treatment (Saw Palmetto; 160 ml/kg/day and 320 ml/kg/day) and standard groups. The selected doses of *N. sativa* oil have been considered as the high dose and maximum dose. Clomiphene citrate have been considered as the standard drug. All animals were induced with PCOS, except the control group, using oral letrozole (1 mg/kg b.w p.o) where the clomiphene citrate at the dose (1 mg/kg b.w p.o) [4].

PCOS induction and treatment

Rats were randomly assigned to three equal groups, whereas Group I is received as animal with by 0.5% w/v of CMC for 46 days, Group II animals as diseased control received only letrozole (1 mg/kg b.w p.o) that is dissolved in 0.5% w/v of CMC administered from 1^{st} to 21^{st} day (21 days). Group III Animals served as test control received letrozole dissolved in 0.5% w/v of CMC from 1^{st} to 21^{st} day (21 days) and 160 mg/kg b.w p.o Saw palmetto from 22^{nd} to 46^{th} day Group IV Animals served as test control received letrozole dissolved in 0.5% w/v of CMC from 1^{st} to 21^{st} day (21 days) and 320 mg/kg b.w p.o Saw palmetto from 22^{nd} to 46^{th} day. Group V Animals served as standard control received letrozole dissolved in 0.5% w/v of CMC from 1^{st} to 21^{st} day (21 days) and 1 mg/kg b.w p.o Clomiphene citrate from 22^{nd} to 46^{th} day (25 days).After the complication of dosing at 46^{th} day. First measurement of heart rate and blood glucose level.

Blood samples will be collected by retro orbital puncture. The collected blood is used for estimation of blood boimaker like glycosylated hemoglobin and serum biomarkers i.e., Total Cholesterol (TC), Triglycerides (TG), HDL-cholesterol (HDL-c), LDL-cholesterol (LDL-c). Following, the animals were sacrificed by ketamine overdose and measurement of uterine weight and length of uterus and the ovary specimen was collected and used for histopathology and for tissue biochemical estimation of Superoxide Dismutase (SOD), catalase and glutathione activity were done [5].

Body weight measurement: After initiation of the study, once in a week body weight of every rat was measured by digital weighing machine and the difference of the body weight was noted.

Measurement of heart rate: An overnight fasted rats was anaesthetized with ketamine (80 mg/kg, i.p. and xylazine (16 mg/ kg, i.p.). The reflexes of the animal are checked and it is placed on a suitable rodent surgical table or a flat

unmovable surface. Sample was collected by retro orbital puncture in rat and processed to obtain serum and tissue collection.

Biochemical analysis

Serum parameters: The blood sample (2 ml) was used to separate serum. It was obtained by allowing the blood samples to clot then centrifugation at 3000 rpm for 20 minutes and kept at (-20°C) and used to measure levels of, Total Cholesterol (TC), Triglycerides (TG), High Density Lipoproteins Cholesterol (HDL-C), Very Low Density Lipoproteins Cholesterol (VLDL-C) and Low Density Lipoproteins Cholesterol (LDL-C) [6].

OGTT (Oral Glucose Tolerance Test): On day 46 of study period OGTT was performed, the initial fasting blood samples were withdrawn by tail vein puncture technique (for FBG) that gives the '0 h' value for blank OGTT. Each rat received orally 0.5 ml solution containing 400 mg glucose and the blood samples were withdrawn at 2 h after glucose administration to get the OGTT pattern of rats. The blood sugar level was measured using glucometer and the blood sugar level was recorded in mg/dl [7].

Antioxidant enzymes and protein levels were estimated in ovarian tissue of control and treated animals. ROS value was determined using the protocol used. Catalase (CAT), levels were analyzed as described in Chance and Maehly with some modifications. Superoxide Dismutase (SOD) was assessed by the protocol and reduced Glutathione (GSH); measured using UV spectrophotometer [8].

Measurement of ovary weight at the end of study on 46 day, all the animals were sacrificed and ovary were removed, cleaned from fats and subjected to gross examination and later weighted. The ovaries were dissected and right ovaries were frozen immediately at -70°C until used for determination of oxidative stress markers whereas, left ovaries were used for histopathological examination.

Measurement of serum lipids profile: TC, TG and HDL-c are analysed by semi auto analyser by using the erba kits where the TC, TG, HDL-C the absorbance of the test and standard was measured against blank at 505 nm (500 nm-540 nm) auto analyzer using reagent blank. The concentration of triglycerides (mg/dL) in the serum sample was recorded directly from the auto analyzer. LDL-c and VLDL-c were measured by using Friedewald's formula.

LDL Cholesterol=Total Cholesterol-(HDLc-VLDLc)

where VLDLc=TG/5

Histopathological examination: The ovaries were dissected, fixed in 10% buffered formalin for 6 hours at room temperature and washed in a phosphate buffer saline solution. The fixed tissues were dehydrated in an ascending series of ethanol, cleared in xylene then embedded in paraffin. 5 µm thick sections were mounted in slides previously treated with 3-aminopyropyl triethoxysilane and stained with hematoxylin and eosin for light microscopy.

Statistical analysis: All the values were expressed aa mean ± SEM. Statistical comparison were performed by one way ANOVA followed by Dunnett compare versus control column using Graph Pad Prism version 5.0. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ was considered as significant compared to disease control [9].

RESULTS

Anthropometric parameters (Body, ovarian and uterine weights)

As presented, body weight and ovary weight were markedly increased ($P<0.0001$) while uterine weight were notably decreased ($P<0.001$) in the PCOS group. Both Saw Palmetto and Clomiphene Citrate significantly reduced in body weight and ovary weight ($P<0.0001$-0.001) with significant increase in uterius weight ($P<0.0001$-0.001) compared to the PCOS groups [10].

Oral Glucose Tolerance Test (OGTT)

On the final day of experiment blood glucose levels were measured. Mean ± SEM is glucose levels were again measured were markedly increased ($P<0.05$) as compared to treated group. PCOS and clomiphene citrate group depicted significant increase ($P<0.05$) when compared with control group, whereas, Saw palmetto treated groups illustrated significant increase ($P<0.05$) as compared to control group (Figure 1).

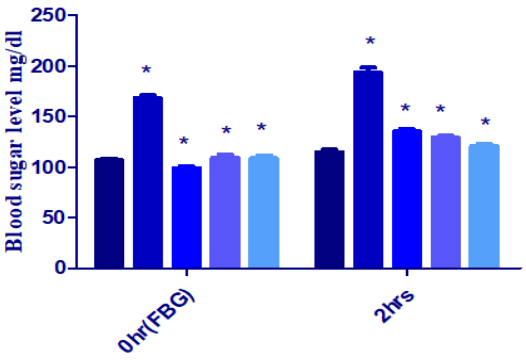

Figure 1 Effect of saw palmetto on OGTT in letrazole induced PCOS. Note: : Normal vehicle; : Letrazole; : Letrazole+Saw P 160 mg; : Letrazole+Saw P 360 mg; : Letrazole+Clomiphene C

Measurement of ovary weight at the end of study on 46 day, all the animals were sacrificed and ovary were removed, cleaned from fats and subjected to gross examination and later weighted. The ovaries were dissected and right ovaries were frozen immediately at -70°C until used for determination of oxidative stress markers whereas, left ovaries were used for histopathological examination. As per data on the PCOS induced rats (Group-II) has shown significant increased (193.5 ± 8.732) in OGTT of when compared with normal control (114.7 ± 4.437 Group-I). Group-III and Group IV rats treated with Saw palmetto have shown significant decreased (135.0 ± 4.636), (129.2 ± 3.269) in OGTT when compared with Group-II rats [11].

Effect of saw palmetto on R-R interval, heart rate in letrazole induced PCOS

R-R intervals were markedly increased (P<0.0001) while heart rate were notably decreased (P<0.0001) in the PCOS group. Both saw palmetto and clomiphene citrate significantly reduced in R-R intervals (P<0.0001) with significant increase in heart rate (P<0.0001) compared to the PCOS groups [12].

Saw palmetto enhanced serum parameters status in letrozole-induced PCOS rats

Serum TC, TG, VLDL-C and LDL-C levels were markedly increased (P<0.001) while HDL-C were notably decreased (P<0.001) in the PCOS group. Both Saw Palmetto and clomiphene citrate significantly reduced TC, TG and LDL-C levels (P<0.001-0.01) with significant increase in HDL-C level (P<0.001-0.01) compared to the PCOS group.

Saw palmetto enhanced ovarian antioxidant status in Letrozole-induced PCOS rats

PCOS group exhibited significant (P<0.0001-0.001) decrease in ovarian CAT, SOD and GSH activities when compared to the control group (Tables 1-5). Treatment with Saw Palmetto significantly (P<0.05) increased the ovarian CAT, SOD and GSH activities in a dose-dependent manner compared with the PCOS group [13].

Table 1 Effect of saw palmetto, on anthropometric parameters: Body weight, ovary weight and uterine weight in letrozole-induced PCOS rats

Parameter/group		Group I (Control)	Group II (PCOS)	Group III (PCOS+SP 160 mg)	Group IV (PCOS+SP 320 mg)	Group V (PCOS+CC)
BW	**Initial (gm)**	142.0 ± 10.89	101.6 ± 23.62	142.0 ± 13.30	138.5 ± 23.48	138.5 ± 7.065
	Final (gm)	179.3 ± 16.11	144.3 ± 35.69	168.5 ± 11.39	160.5 ± 27.60	149.8 ± 7.009
	% change	20.61 ± 4.062	29.05 ± 4.402	15.81 ± 3.361	13.62 ± 3.550	7.575 ± 0.960
Ovarian wt (mg)		30.00 ± 5.773	63.33 ± 7.453	41.66 ± 6.871	33.33 ± 4.714	46.66 ± 7.453
Uterine wt (mg)		190.0 ± 12.90	145.0 ± 12.58	300.0 ± 22.36	325.0 ± 18.02	236.6 ± 23.57***
Note: ***Saw palmetto significant for P<0.05						

Table 2 Effect of saw palmetto, on oral glucose tolerance test in letrozole-induced PCOS rats

Group	Gp Name	Altered glucose metabolism	
		0hr (FBG)	2 hrs
I	Control	106.5 ±3.354	114.7 ± 4.437
II	PCOS	168.2 ± 4.656	193.5 ± 8.732
III	PCOS+S P 160 mg	99.0 ± 3.000	135.0 ± 4.636
IV	PCOS+S P 320 mg	109.0 ± 5.477	129.2 ± 3.269
V	PCOS+CC	108.7 ± 5.309	120.5 ± 4.03

Table 3 Effect of Saw Palmetto, on R-R interval and heart rate in letrozole-induced PCOS rat

Parameter/group	Group I (Control)	Group II (PCOS)	Group III (PCOS+SP 160 mg	Group IV (PCOS+SP 320 mg)	Group V (PCOS+CC)
R-R interval	0.206 ± 0.001	0.301 ± 0.009	0.251 ± 0.004	0.225 ± 0.003	0.212 ± 0.001
Heart rate (beats/min)	291.3 ± 2.213	199.3 ± 6.308	239.1 ± 3.840	266.4 ± 3.606	283.0 ± 2.512

Table 4 Effect of Saw Palmetto on Serum levels in letrazole induced PCOS rats

Parameter/group	Group I (Control)	Group II (PCOS)	Group III (PCOS+SP 160 mg)	Group IV (PCOS+SP 320 mg)	Group V (PCOS+CC)
TC (mg/dl)	59.58 ± 8.253	121.17 ± 1.832	96.65 ± 8.950	90.65 ± 16.16	70.93 ± 6.824
TG (mg/dl)	308.94 ± 16.26	556.08 ± 11.10	456.83 ± 62.08	472.76 ± 43.19	439.02 ± 32.52
HDL-C (mg/dl)	37.54 ± 2.343	17.01 ± 1.401	21.82 ± 0.738	27.53 ± 2.064	33.02 ± 2.281
VLDL-C (mg/dl)	61.78 ± 3.253	111.37 ± 2.254	91.36 ± 12.41	94.55 ± 8.637	87.79 ± 6.503
LDL-C (mg/dl)	83.81 ± 9.557	215.56 ± 3.438	166.19 ± 15.48	157.67 ± 20.28	125.70 ± 12.95

Table 5 Effect of saw palmetto, on antioxidant status in letrozole-induced PCOS rats

Parameter/group	Group I (Control)	Group II (PCOS)	Group III (PCOS+SP 160 mg)	Group IV (PCOS+SP 320 mg)	Group V (PCOS+CC)
TC (U/mg protein)	34.56 ± 0.873	15.56 ± 1.527	20.62 ± 1.382	24.81 ± 0.953	29.43 ± 1.452
SOD (U/mg protein)	0.108 ± 0.004	0.069 ± 0.016	0.087 ± 0.003	0.095 ± 0.001	0.098 ± 0.003
GSH (U/mg protein)	40.26 ± 0.150	35.13 ± 2.702	41.22 ± 1.935	41.50+2.207	47.11+2.062

Histopathology of rats' ovary

Normal control: Section of ovary from normal group rat showing presence ovarian cortex with many follicles of different stages of development, unilayer Primary Follicles (uPF), multilayered Primary Follicles (mPF) and

Secondary Follicles (SF). The follicles are separated by a few stromal interstitial cells (S). Some Oocytes (Oc) appear in some follicles. An Antral mature graffian Follicle (ArF) shows Granulosa cells (Gc), an oocyte surrounded with corona Radiata cells (Cr) and many layers of Cumulus oophoros (Co).

The follicle shows one large Follicular cavity (Fcv) filled with Liquor folliculi (Lof) and surrounded by Theca Interna (ThI) and Theca Externa cells (ThE). A Corpus Luteum (CL) with outer layers of Theca Lutein cells (ThL) and inner Granulose cells (GL); Secondary Follicle (SF), Primary Follicle (PF) (Figure 2) [14].

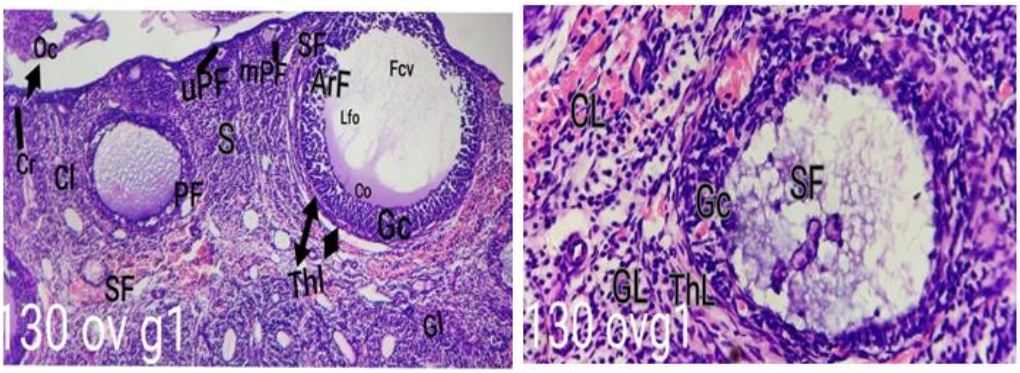

Figure 2 Histopathological studies of normal rat ovary

Negative control: Section of ovary from PCOS rat showing Cortex with many Follicular cysts (Fcy) of different shape and wall thickness that contain acidophilic materials (stars) in their lumens and are separated by highly cellular interstitial stroma (S). Higher magnification of figure showing two follicular cysts; the wall of one cyst (Fcy1) is thick and formed mainly of Theca interna cells (ThI) and the wall of another cyst (Fcy2) is folded, thin and formed of one layer of granulosa cells surrounded by a thick layer of theca cells.

Many follicular cysts (Fcy) with no oocytes that have thick walls formed mainly of theca interna cells. Note the numerous congested blood vessels (V) in the cortex. The higher magnification of Fcy1 shows that it has thin walls which are formed by a single layer of degenerated Granulosa cells (Gc), surrounded by thick layers of Theca cells (Thc) showing signs of luteinization and filled with amorphous Liquor folliculi (Lfo); Medulla (Med), Blood Vessel (V) (Figure 3) [15].

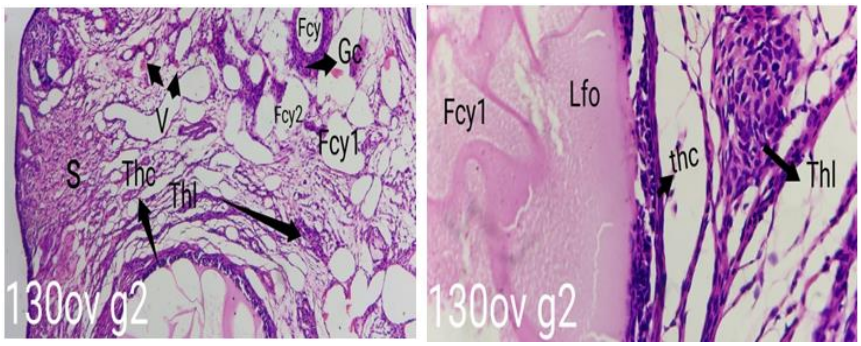

Figure 3 Histopathology of PCOS treated rat ovary

Test control at medium dose (160 mg): Section of ovary from PCOS rat showing ovarian cortex with many follicles in different stages of development; Primary Follicles (PF) and Primordial Follicles (PrF) containing an Oocyte (Oc) in its lumen. Note the little amount of interstitial Stroma (S) when compared to that of PCO. Many growing (Gf) and Primordial follicles. Both have thick walls formed of many layers of Granulosa cells (Gc), theca interna (ThI) and Theca externa layers (ThE). An Oocyte (Oc) surrounded by zona pellucida (arrow) and little stroma (S) are seen. C-two corpora Lutea (CL) with small lipid droplets (arrows) in their Granulosa (GL) and Theca lutein (ThL) cells. One Growing Follicle (Gf) is apparent with some vacuolations (curved arrows) of its Follicular cells (Fc) (Figure 4) [16].

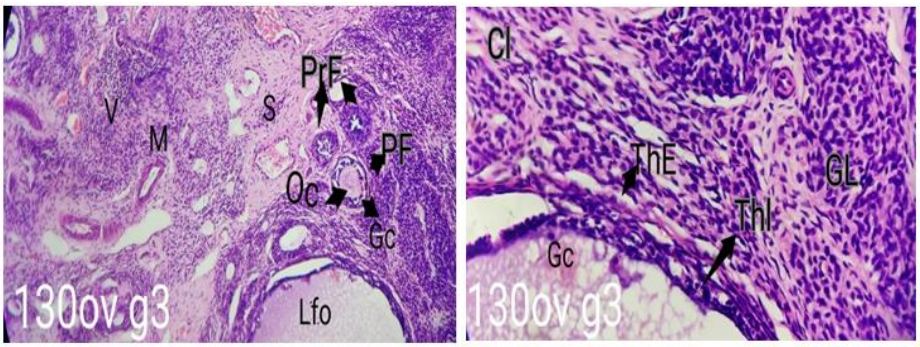

Figure 4 Histopathological studies of Saw P dose at 160 mg treated rat ovary

Test control at high dose (320 mg): Section of ovary from PCOS rat showing nearly normal ovarian cortex with many follicles in different stages of development; multilayered Primary Follicles (mPF) and primordial follicles containing an Oocyte (Oc) in its lumen. Many Growing (Gf) and Primordial follicles. Both have thick walls formed of many layers of Granulosa cells (Gc), Theca Interna (ThI) and Theca Externa layers (ThE). An Oocyte (Oc) surrounded by zona pellucida (arrow) and little stroma (S) are seen. Two Corpora lutea (CL) with small lipid droplets (arrows) in their Granulosa (GL) and Theca Lutein (ThL) cells. One Growing follicle (Gf) is apparent with some vacuolations (curved arrows) of its Follicular cells (Fc) (Figure 5) [17].

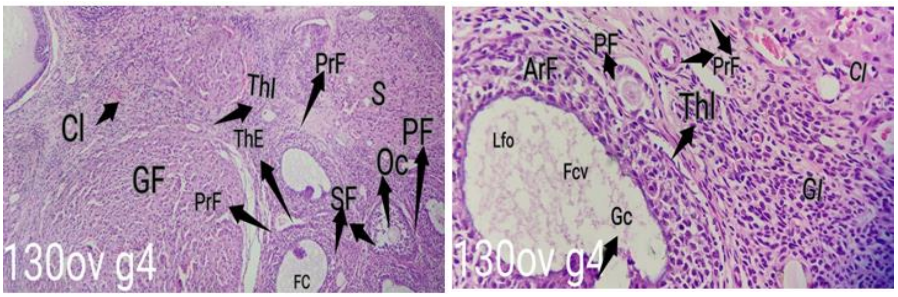

Figure 5 Histopathological studies of Saw P dose at 320 mg treated rat ovary

Standard control: Section of ovary from PCOS rat showing a nearly normal ovarian cortex with many follicles in different stages of development; multilayered Primary Follicles (mPF) and Secondary Follicles (SF) containing an Oocyte (Oc) in its lumen where The follicles are separated by a few stromal interstitial cells (S). An antral mature Graffian Follicle (ArF) shows Granulosa cells (Gc). Many Growing (Gf) and Primoridal Follicles (pfF). Both have many layers of Granulosa cells (Gc), Theca Interna (ThI) and Theca Externa layers (ThE). An Oocyte (Oc) surrounded by zona pellucida (arrow) and little Stroma (S) are seen. C-Two Corpora Lutea (CL) with small lipid droplets (arrows) in their Granulosa (GL) and Theca Lutein (ThL) cells. One Growing follicle (Gf) is apparent with some vacuolations (curved arrows) of its follicular cells (Fc) (Figure 6).

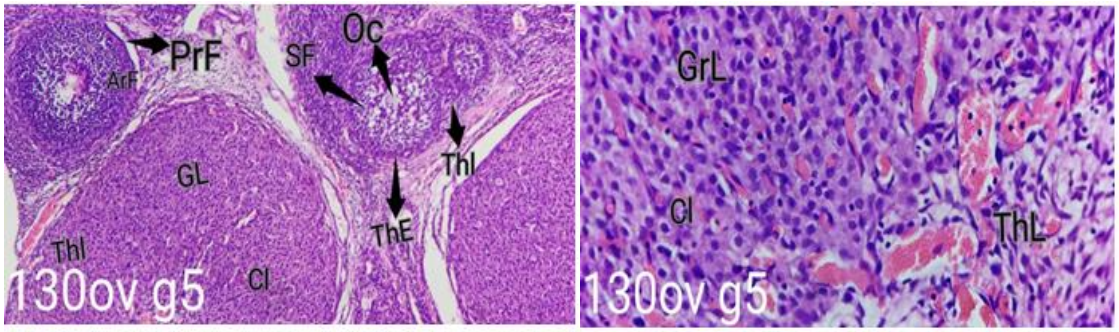

Figure 6 Histopathological studies of clomiphene C treated rat ovary

DISCUSSION

Polycystic Ovary Syndrome (PCOS) is a female reproductive and endocrine disease that results in follicular development and ovulation disordes due to steroid hormone imbalances. The basic clinical and pathological features of PCOS are chronic persistent anovulation and hyperandrogenic, bilateral cystic enlargement of the ovaries (large than normal ovaries in all follicle periods, including preantral) and significally increased androgen levels with realtively insufficient estradiol in the peripheral blood. Studies have found that increased androgen levels or hyperandroogenism, constitute a core feature of PCOS-related reproductive and endocrine disorder. A deficiency in activity of aromatase was one intraovarian disturbance in steroidogenesis thought to cause PCOS. Due to aromatase catalyzes the rate-limiting step in biosynthesis of estrogen to androgens, decrease in activity of this enzyme could be expected to result in increased ovarian androgen production and development of PCOS.

In the present study period the control animal maintained consistent levels of body weight, varying in normal range. In the study Letrazole treated rats (Group II) showed progressive and significant increased in body weight as compared to the normal Group animals. This may be due to the excess amount of 'bad' estrogen circulating in the blood stream. There are various from of estrogen. The three most researched and understood estrogen from are 2-hydroxy ("good") estrogen and hydroxyl and 16-hydroxy (both "bad"). Excessive amounts of bad estrogen may get stored in adiposities (depositions of abdomimal fat). Since fat cells contain the aromatase enzyme, so testosterone increasing converted on to estrogen by aromatase enzyme that causes increased production of estrogens. When there is too much "bad" estrogen and not enough progesterone can be synthesized to counteract its effects that stimulation is called as estrogen dominance. Estrogen predominance can leads to weight gain and both are the primary factor for ovarian cyst.

The PCOS induced rats when treated with saw palmetto 160 mg and Saw palmetto 320 mg have shown significant decreased in the body weight as compared to group-rats. This effect may be due to Saw Palmetto induced improved estrogen metabolism by altering liver function, in a way which increases the rate at which estrogen are metabolized in to inactive from, mainly converted bad estrogen into inactive from thus clearing excess estrogen from the body. Whereas the Group-V rats shown significant decreased in the body weight when compared with the letrazole administered rats (Group-II). It may be due to antiestrogen effect of clomiphene citrate. After administered of CC its leads to depletion of estrogen receptors at the level of the pituitary and hypothalamus, thus interrupting the negative feedback that estrogen normally produces. As a results GnRH secretion is improved that stimulates pituitary to produce Follicle Stimulating Hormone (FSH), which causes follicular growth and maturation with emergence of one or more dominant follicles.

Effect on ovarian weight

As per the study results rats treated with letrazole (Group-II) showed increased ovarian weight as compared to the normal control, because letrazole arrest the production of Follicle Stimulating Hormone (FSH). The suppression of FSH causes only partial development of ovarian follicle. So, there is less chance to produce mature egg from ovaries. Deposition of those immature eggs in the ovarian follicles causes follicular cysts that may cause thickening

of ovarian capsule and hyperplasia of the theca interna cells in the ovary, but Group-V rats have shown decreased ovarian weight due to the antiestrogen effect of C.C.Group III and Group IV treated rats also have shown decreased ovarian weight. This effect may be due to improved secretion of FSH from hypothalamus pituitary axis because FSH is solely responsible for proper stimulation of ovarian follicular, which can produce mature egg during ovulation.

Effect on uterine weight

The rats treated with letrazole (Group-II) showed decreased in uterine weight as compared to the normal control. Reduction in the uterine weight is mainly is mainly associated to the atrophy of organ and in the present study letrazole showed development of polycystic ovary due to the some abnormal hormonal interactions. As per, an ovary with full of cyst dose not ovulate in normal way that causes degenerative changes in the uterine wall, but as because Group-III, Group-IV and Group-V treated rats showed normal ovulation process after treatment so, that there is less chances of degenerative changes in the uterine wall, that's the reason behind of the Group-III, Group-IV and Group-V showed increased uterine weight as compared to group-II.

Effect on Oral Glucose Tolerance Test (OGTT)

The glucose levels increased significantly when measured in the PCOS induced letrazole (Group-I) as compared with normal control (Group-I). Letrozole seems to create a disturbance in the hormonal profile of the animals mainly because of increased androgen levels. This increased androgen levels in turn induced insulin resistance hence creating a decreased glucose tolerance. Treatment of Saw palmetto (Group-III, Group-IV) treated rats shows significant decreased in glucose level when compared with the Group-II. Clomiphene Citrate (Group-V) treated rats which shows significant decreased in glucose level when compared with the Group-II. Group-III, Group-IV and group reduced the glucose levels significantly as compared to PCOS group. This may be due to the fact that metformin treatment decreased the glucose resistance by maintaining the glucose homeostasis and also by improving the insulin-mediated uptake of glucose.

Effect on Heart Rate (HR)

The letrazole induced PCOS (Group-II) rats there was a significant decrease in total power (TP) and high frequency power (HF), the low frequency power LF and ratio of LF/ HF was significantly increased when compared to control (Group-I). Where treatment drug Saw Palmetto (Group-III, Group-IV) showed the TP and HF was significantly increased, the LF and LF/HF ratio was significantly decreased when compared to Group-II. This study shows that intensive Low frequency Saw palmetto (Group-III, Group-IV) treated rats modulates the autonomic activity of heart by altering the HR, total power TP and LF/HF ratio in rats with Letrazole induced PCOS (Group-II). A variety of responses can be appreciated in the endocrine, metabolic and nervous system through intramuscular needle insersion and stimulation, which produces a specific pattern of afferent activity in peripheral nerve fibers. In a rodent model of PCOS the acupuncture has sympathetic depression effect on the central nervous system and reduced ovarian sympathetic tone.

Effect of serum TC, TG, HDL-C, VLDL-C and LDL-C

The most common lipid abnormalities in PCOS are hypertriglyceridemia and hypercholesterolemia. Higher levels of cholesterol (Hypercholesteremia) and higher levels of triglyceride (Hypertriglyceridemia) are the primary factor involved in the escalation of coronary heart disease and atherosclerosis, the secondary complication occurring in PCOS. Hyperandrogenism also lead to dyslipidemia free testosterone levels have been implicated in lowering HDL cholesterol levels. Androgens through the interaction with Androgen Receptors (AR) decrease the catabolic removal of LDL. More over in the study Saw Palmetto (Group-III, Group-IV) and Clomiphene citrate (Group V), dyslipidemia manifested by significant increases in total cholesterol, triglyceride, VLDL, LDL as compared with the letrazole Induced (Group II) and Group-III, Group-IV and Group-V significant decreases in HDLc as compared with the Group-V. The is probably due to excessive hypersecretion of apolipoprotien B and Very Low Density Lipoprotein (VLDL) from the following insulin stimulation, ultimately resulting in hpertriglyceridaemia, low levels of High-Density Lipoprotein (HDL) and high level of low-density lipoprotein (LDL). Letrazole treated groups showed a marked reduction in plasma triglyceride, cholesterol and LDL cholesterol levels along with a significant increase in HDL cholesterol levels as compared to PCOS-positive rats.

Effect on Catalase (CAT), Superoxide Dismutase (SOD), reduced Glutatione (GSH)

Antioxidant enzyme provides the first line defence mechanism which prevents biological molecule (lipids, protein, DNA) from damage by inhibiting ROS formation. Hydrogen peroxide (H_2O_2), Nitric Oxide (NO), superoxide anion (O_2) and hydroxyl radical (OH) are central reactive oxygen and nitrogen species that are involved in tumorigenesis and mutagenesis. Superoxide Dismutase (SOD) counteracts toxic effects of superoxide anion. Levels of antioxidant enzyme are important because dismutation of superoxide anion to from H_2O_2 is catalysed by SOD while H_2O_2 is converted to water molecules by the activity of Catalase (CAT) while reduced glutathione (GSH) is used as an election donor in such reaction where similarly, GSH levels are retained by thiol containing non-protein compound called glutathione reductase (GSH). Rats treated with letrazole (Group-II) showed decreased levels of CAT, SOD, GSH (markers of antioxidant potential) when compared with normal control (Group-I), treatment of Saw palmetto (Group-III, Group-IV) treated rats shows significant increase in the CAT, SOD, GSH activity when compared with the Group-II. Clomiphene Citrate (Group-V) treated rats which shows significant increase in the CAT, SOD, GSH activity when compared with the Group-II. Increased oxidant levels may alter the stereo diagnosis in ovarian contributing to increased androgen production and polycystic ovaries. In the present study, it was observed that the PCOS animals exhibited elevated oxidative stress markers and reduced endogenous antioxidants in ovary.

SOD<catalase and GSH activity were significantly diminished in the PCOS group and concomitant treatment with Saw palmetto restored their activites. This is in unison with earlier reported antioxidant activity of saw palmetto.

Histopathological study of rats' ovary

Control ovarian cortex showed many follicles at different stages of development; unilayer primary follicles, multilayer primary follicles and secondary follicles with few stromal interstitial cells. An antral follicle showed granulosa cells, an oocyte with corona radiata and many layers of cumulus oophoros. Mature graffian follicles with large cavities filled with liquor folliculi and surrounded with theca interna and theca externa cells were also observed. A corpus luteum with an outer layer of theca lutein cells and inner granulose cells was seen in the normal control treated Group-I. In the PCO group, the cortex showed many follicular cysts of different shape and wall thickness that contained acidophilic materials in their lumens and were separated by highly cellular interstitial stroma. Higher magnifications showed thick walled follicular cysts formed mainly of theca interna cells. Some cysts were folded, of thin walls and formed of one layer of granulosa cells surrounded by a thick layer of theca cells.

Many follicles with no oocytes and numerous congested blood vessels in between were observed Large cystic antral follicles filled with amorphous liquor folliculi showed degenerated granulosa cells and thick layeredtheca cells with signs of luteinization in the negative control letrazole induced (Group-II). On the other hand, where treated drug Saw P 160 mg (Group-III) , Saw P 360 mg (Group-IV) and Std drug clomiphene C (Group-V) rats showed nearly normal ovarian cortex with many follicles in different stages of development, multilayer primary follicles and secondary follicles (Primoridal follicles) with oocyte in lumen. A small amount of interstitial stroma was observed when compared to that of PCO. Many growing and secondary follicles with thick walls formed of many layers of granulosa cells, theca interna and theca externa layers, containing oocytes surrounded by zona pellucida, in addition to a low level of stroma were seen. Corpora lutea with small lipid droplets in their granulosa and theca lutein cells and one growing follicle with some vacuolations of its follicular cell were also seen.

CONCLUSION

PCOS is a multifaceted endocrine and metabolic syndrome with anovulation. The etiology of PCOS still remains unclear. Due to its reflecting complicated multiple pathophysiological mechanisms, the exact treatment method is unknown and many of the available pharmacological treatments for PCOS also have lots of side effects and they are more expensive too. To understand the clear-cut mechanism and treatment of PCOS, multiple animal and human model studies may be needed. In the present study, we used the administration of Saw Palmetto (Saw P) powder from showed inhibits 5α-reductase or has anti-androgen activity the letrazole induced PCOS in rats was confirmed by following estimation.

Physical parameters

The Saw Palmetto treated rat exhibited significant decreased in body weight when compared to the diseased control (PCOS induced), because androgen predominance can leads to weight gain and both are the primary factor for ovarian cyst. So, the significant reduction of body weight is an indication of antiestrogenic properties of saw palmetto. Saw palmetto treated rats also showed significant decreased in ovarian weight and increased in uterine weight compared to PCOS induced group, the decreased in ovarian weight is mainly due to improved secretion of FSH from hypothalamus-pituitary axis because FSH is solely responsible for proper stimulation of ovarian follicles, which can produce mature egg during ovulation and increased ovarian weight is an indication that there is no such degenerative changes in the uterine wall. From this property we can conclude that Saw Palmetto also improved fertilization of mature egg that helped in ovulation.

Oral Glucose Tolerance Test (OGTT): Saw Palmetto treated rat showed significant decreased in blood sugar as compared to PCOS treated rats. Because of its antihyperglycemic effect and caused a decrease in the glycaemic levels by conceivably playing its role as potentiating the insulin secretion by the β-cells of Islets of Langerhans thus promoting a balanced uptake of glucose by the cells . It also suggested that Saw Palmetto might be a potential agent for glycaemic control through enhancement of insulin-dependent receptor kinase activity, thus inducing the insulin signalling pathway and consequently causing increased glucose transporter 4 translocation and increased glucose uptake.

Heart rate: Polycystic ovary syndrome is a state of increased sympathetic activity which may have associated with the metabolic disorder such as CVD and reproductive disturbances. This study concludes that low frequency Saw

Palmetto has a significant effect on PCOS rats, which reduces the HR and sympathetic activity. This non pharmacological less expensive alternative therapy can be implemented for PCOS to reduce the cardiovascular risk in patients.

Biochemical parameters: Saw Palmetto significantly improved the lipid profile by reducing the serum levels of TC, TG, LDL-C, VLDL-C and increasing HDL-C in PCOS rats. It has been shown that the letrozole model of PCOS develops dyslipidemia and disrupted redox balance similar to human PCOS. Dyslipidemia associated with letrozole-induced PCOS manifests with elevated TC, TG, LDL-C, VLDL-C and reduced HDL-C or at least one of them in other studies. Studies that have reported no change in lipid profile after letrozole administration, at this point it is concluded that saw palmetto also posses antihyperlipidemic activity.

Tissue parameters: Many studies reported oxidative stress as one of the pathological factor for PCOS. Increased oxidant levels may alter the stereo diagnosis in ovaries contributing to increased androgen production and polycystic ovaries. In the present study, In case of diseased group there will be decreased in scavenging enzyme activities such as Super Oxide Dismutase (SOD), Catalase (CAT) and it was observed that the PCOS animals exhibited elevated oxidative stress markers and reduced endogenous antioxidants in ovary. SOD, Catalase and GSH activity were significantly diminished in the PCOS group and concomitant treatment with saw palmetto restored their activities. The saw palmetto is reported as restore the activity of both these antioxidant enzymes and possibly could reduce generation of free radicals.

Histopathology of ovary: Histopathology section of ovary showed that the animals treated with Saw palmetto showed formation of oocyte and follicles showed normal granulosa layer with defined thecal layer as compared to the letrazole induced group of rat.

REFERENCES

[1] Catteau-Jonard S, et al. Anti-Mullerian hormone and polycystic ovary syndrome. *Gynecol Obstet Fertil*. 39(9), 2011: 514-517.
[2] WHO and Geneva. WHO methods and data sources for global burden of disease estimates 2000-2011. *Health Stat Informat Syst*. 2013.
[3] Diamanti-Kandarakis E, et al. Pathophysiology and types of dyslipidemia in PCOS. *Trends Endocrinol Metabol*. 18(7), 2007: 280-285.
[4] Kenna HA, et al. Reproductive and metabolic abnormalities associated with bipolar disorder and its treatment. *Harvard Rev Psychiatr*. 17(2), 2009: 138-146.
[5] Aktas A, Nergiz Y and Akkus M. The effect of valproic acid on rat ovarium and the protective role of vitamin E and folic acid: An ultrastructural study. *Afr J Biotechnol*. 9(34), 2010.
[6] Jayasena CN and Franks S. The management of patients with polycystic ovary syndrome. *Nat Rev Endocrinol*. 10(10), 2014: 624-636.
[7] Farmer JB and Levy GP. A simple method for recording the electrocardiogram and heart rate from conscious animals. *Br J Pharmacol Chemother*. 32(1), 1968: 193-200.
[8] Daneasa A, et al. Spironolactone and dimethylsulfoxide effect on glucose metabolism and oxidative stress markers in polycystic ovarian syndrome rat model. *Experim Clin Endocrinolo Diabet*. 122(3), 2014: 154-162.
[9] Hayashi I, et al. High-throughput spectrophotometric assay of reactive oxygen species in serum. *Mut Res Genet Toxicol Environ Mutagen*. 631(1), 2007: 55-61.
[10] Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. *Analyt Biochem*. 27(3), 1969: 502-522.
[11] Friedewald WT, et al. Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 18(6), 1972: 499-502.
[12] Baravalle C, et al. The role of ACTH in the pathogenesis of polycystic ovarian syndrome in the rat: Hormonal profiles and ovarian morphology. *Physiol Res*. 56(1), 2007: 67-78.
[13] Jahan S, et al. Ameliorative effects of rutin against metabolic, biochemical and hormonal disturbances in polycystic ovary syndrome in rats. *J Ovarian Res*. 9(1), 2016: 1-9.
[14] Ramadoss M, et al. Heart Rate changes in electroacupuncture treated polycystic ovary in rats. *J Clin Diagn Res* 10(3), 2016: CF01.
[15] El-Bahaie EM, et al. Reversal of haemostatic changes in letrozole-induced PCOS rat model with vitamin D supplementation. *Med J Cairo Univers*. 87, 2019: 1753-1767.

[16] Ullah A, et al. Protective effects of GABA against metabolic and reproductive disturbances in letrozole induced polycystic ovarian syndrome in rats. *J Ovarian Res*. 10(1), 2017: 1-8.
[17] Attia GM, et al. Metformin decreases the expression of VEGF-A and CTGF-1 and improves histological, ultrastructural and hormonal changes in adult rat polycystic ovary. *Folia Biol*. 67(1), 2019: 25-43.