Studying the Effectiveness Low Carbohydrate Diet and Anise Herb on Female Rats with Polycystic Ovary Syndrome

Amr A. Rezq[1], Dina H. Abdel Kader[2] Alaa O. Aboraya[1] and Moreen A. Adly[1]

[1] Nutrition, Nutrition and Food Sciences Dept., Faculty of Home Economics-Helwan University- Egypt
[2] Histology and Cell Biology - Cairo University

Abstract

Polycystic Ovary Syndrome (PCOS) is one of the most common hormonal disorders among women of reproductive age.Therefore, the current study was performed to discover out the effect of low carbohydrate diet ((LCD) and anise herb on PCOS - caused by the administered of letrozole (1 mg/kg p.o.) for 21 days in female rats. The results showed a significant decline ($p<0.05$) in body weight gain (BWG), changes in BWG % of PCOS rats feeding on LCD alone and the supplemented-LCD with anise, compared to that fed on the basal diet (BD) alone and the supplemented-BD with anise. In comparison to the positive control group, PCOS-rats fed on the either LCD alone or the supplemented-BD or -LCD with anise have significant ($P < 0.05$) amelioration in the serum levels of blood glucose, TC, TG, TL, LDL-c, VLDL-c, HDL-c, as well as insulin, luteinizing, follicle stimulating, testosterone, estradiol, progesterone, ehydroepiandrosterone sulfate and prolactin hormones, and activates of antioxidant enzymes (CAT, SOD, GSH and GPx). A better improvement in serum levels of the above parameters and histopathology examination of the ovary was discovered at feeding PCOS-rats on LCD alone or supplemented-LCD with anise, compared to the other groups. Finally, the ability of LCD and anise to enhance the treatment of PCOS degeneration makes it a hopeful natural diet and herb for the management of PCOS.

Keywords: Sex Hormones; Lipid Profile; Polycystic Ovary Syndrome; Low Carbohydrate Diet.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is one of the most common hormonal disorders among women of reproductive age, with a global prevalence ranging from 4% to 20% **Pundir *et al*., (2020) and Liu *et al*., (2021)**. Such a large spread in the frequency of PCOS reflects the heterogeneous nature of this complex disorder that merges genetic, environmental, endocrine, and behavioral factors. Also, the syndrome is poorly understood and remains underdiagnosed, and female patients are diagnosed with a delay. However, the primary PCOS clinical manifestations are derived from the excess of androgens, absence of ovulation (anovulation), polycystic ovarian morphology, lack of or scanty, irregular menstrual bleedings, acne—an inflammatory condition of the skin in which the skin's sebaceous glands become clogged and infected, excessive growth of dark or coarse hair in a male-like pattern-face, chest and back (hirsutism). The secondary manife-stations include multiple metabolic, cardiovascular, and psycholo-gical disorders **Joham *et al*., (2020**). All this creates serious problems, where the complexity of PCOS requires a quick diagnosis and the development of therapeutic strategies for long-term health issues known as PCOS-related complications, such as insulin resistance (IR) among the metabolic abnormalities. PCOS characteristically evolves with age, from a reproductive disease to a more metabolic disorder with increased incidence of type 2 diabetes and cardiovascular disease in later life **(Joham *et al*., 2020 and(Louwers *et al*., 2020).**

A return to nature and the use of drugs of plant and natural origin takes place in a situation where modern man, by promoting the use of chemical drugs, has faced the side effects and complications of these drugs. Scientific studies have demonstrated the effectiveness and safety of several complementary medicine methods including herbs in the treatment of some diseases **(Rashidi *et al*., 2012).**

Anise or anisum with the scientific name *Pimpinella anisum* L. belongs to the Apiaceae (*Umbelliferae*) family and is very similar to

fennel **Das *et al.*, (2021)**. Anise is widely used in the pharmaceutical, food, beverage, and cosmetic industries as a flavoring and preservative due to its aromatic compounds such as terpenes, anisaldehyde, and estragol **Das *et al*., (2021)**. In traditional medicine, anise is used in for the treatment of many diseases due to its antimicrobial and antioxidant properties **(Ghlissi *et al*., 2020).** Anise contains oleoresin, which is a yellowish-green to orange-brown liquid. The major constituents of aniseed oil are trans-anethole (90%), anisketone, anisaldehyde and methyl chavicol. Anethole helps to relieve oligomenorrhea and improve quality of life in women who are undergoing treatment for PCOS. The phenolic ingredients possess phytoestrogenic features, which may play a greater role in the regulation and improvement of menstrual cycles and LH/FSH secretion in women with PCOS and play an important role in relieving PCOS complications (**Sadrefozalayi *et al*., 2014)**. The current study was performed to discover out the effect of low carbohydrate diet and anise herb on PCOS in female rats.

Materials and Methods

Materials:

Rats: Forty adult female albino rats of Sprague Dawley strain weighing 200±10g was purchased from the Laboratory Animal Colony, Ministry of Health and Population, Helwan, Egypt. All rats were housed at a room temperature of 25 ± 2 °C, relative humidity of 50–55% and light/dark cycles (12/12) in the animal house of the Faculty of Home Economics, Cairo, Egypt for one week for acclimatization.

Diet: Casein, cellulose, D-L methionine, vitamins and minerals, constituents were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, soybean milk, and sucrose were obtained from the local market.

Plant Materials: Dried whole Anise was purchased from herbalist shops in Cairo, Egypt and was identified in National Center for Agricultural Research, Cairo, Egypt.

Drug: Letrozole, sold under the brand name Femara was purchased from The Egyptian Company for Pharmaceutical Trade, Cairo, Egypt.

Chemicals and Kits: All chemicals and Kits for the biochemical analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemical, Dokki, Egypt.

Methods:

Preparation of Basal Diet: The basal diet (AIN-93M) will be consisting of protein (14%), corn oil (5%), mineral mixture (3.5%), vitamin mixture (1%), fiber (5%), sucrose (10%), choline chloride (0.25%) and the remainder have corn starch (up to 100%) were mixed and formulated. according to **(Reeves *et al.*, 1993).**

Preparation of Low Carbohydrate Diet: The low carbohydrate diet was composed of carbohydrate (8.77%), protein (59.46%), and fat (31.77%). While, the amount of vitamins, minerals, L-cysteine, choline, and fiber were the same in the two groups, and tert-butylhydroquinone was calculated as 0.002 mg per gram of fat, all based on AIN 93M determinations as described by (**Monteiro *et al*., 2016**).

Induction of Polycystic Ovary Syndrome (**PCOS):** PCOS was caused in female rats with the administered of letrozole at the concentration of 1 mg/kg p.o. dissolved in 1% of carboxmethlycellulose once daily orally (p.o.) for 21 days (**Kafali *et al*., 2004**) After that 21 days, five rats were sacrificed to ensure the inductive of PCOS, while the other 35 rats were used in the study**.**

Experimental Design and Grouping of Rats: Thirty-five of rats were housed in the animal house at the Faculty of Home Economics, Helwan University in wire cages under adjustment environmental

conditions of the temperature (22±4°C), relative humidity (45% to 50%) and light/dark cycle (12/12 hr). The food and water supplies were uninterrupted during the experimental period. Prior to the trial study, rats were kept for a week to acclimatize. Subsequently, all rats were divided into 5 groups ($n = 7$). Groups were labeled as I, II, III, IV, and V as follows:

Group I: kept as a negative rats (- ve group), received vehicle only (1% aqueous solution of carboxmethlycellulose and feed on the normal basal diet

Group II: kept as a positive rats (+ ve group), PCOS- induced rats fed on the normal basal diet.

Group III: PCOS- induced rats fed on the low carbohydrate diet.

Group IV: PCOS- induced rats fed on the supplemented basal diet with 5% anise powder.

Group V: PCOS- induced rats fed on the supplemented low carbohydrate diet with 5% anise powder.

Biological Evaluations: Body weight of the animals were determined every other day. Also, feed intake (FI) was calculated throughout the experimental period (7 weeks).

Blood Collection and Serum Separation: At the end of the experimental period (7 weeks), all rats were fasted overnight before scarifying and blood samples were collected from each rat and centrifuged to obtain the clear serum for biochemical analysis. The ovary of each rats was dissected out, washed with normal saline solution to remove the blood and fixed in 10% neutral formalin for histopathological examination.

Biochemical Analysis: Serum levels of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined using ELISA Kits according to the manufacturing company instruction (Monobind Inc. USA), which were depended on the technique of the quantitative sandwich enzyme immunoassay according to the described methods by **Tietz, (1995) and Rebar *et al.*, (1982)**, respectively. Serum levels of estradiol (E2),

progesterone, ehydroepiandrosterone sulfate (DHEA-S), prolactin and Estrogen were assessed by Gama kits as described by **(Lavaee *et al*., 2021)**

Estimation of Blood glucose and Serum Insulin Levels: At the end of experimental period and after the rats had been fasted for 12 hrs ., blood samples were collected by tail vein of the rats and blood glucose levels were measured immediately by using a single touch Glucometer (Ascensia ENTRUST, Bayer). The serum insulin levels were measured by a sensitive rat insulin radioimmunoassay kit (Diamond Co, Hannover, Germany) according to the described methods by **(Posario, 2010).**

Estimation of Serum Levels of TC, TG, TL, HDL-c, LDL-c and VLDL-c: Serum levels of total cholesterol (TC), triglycerides (TG), total lipid (TL), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c) and very low density lipoprotein cholesterol (VLDL-c) were estimated using commercial reagent kits (Biomed diagnostic, Egypt) as described by **Zollner and kirsch .,(1962), Vassault *et al*., (1986)**, **Hostmark *et al*., (1991), Friedwald *et al*., (1972)** and **Young., (2001),** respectively. Very low density lipoprotein cholesterol (VLDL-C) was calculated using using Friedewald's formula.

VLDL-c (mg/ dL) = TG/5

Estimation of Serum Oxidative Stress Marker: Malondialdehyde (MDA) was assayed quantitatively in serum using the MDA assay kit by a spectrophotometric method. The MDA in the sample reacts with thiobarbituric acid (TBA) to generate a MDA-TBA adduct. The MDA-TBA adduct is quantified colorimetrically (OD = 532 nm). This assay detects MDA levels as low as 1 nmol/well colorimetric ally **(Ohkawa *et al*., 1979)**.

Estimation of Serum Activity of Antioxidant Enzymes: The procedure that is used for the evaluation of CAT activity depends on the reaction of the enzyme with methanol in the presence of an

optimal concentration of H2O2. The formaldehyde produced is measured spectrophotometrically at 540 nm as described by **Wheeler *et al.*, (1990).**

The standard technique to assay the activity of SOD is that the kits used use an enzyme linked immunosorbent assaydouble antibody principle. The color change is measured spectrophotometrically at 450 nm as described by **Wheeler *et al.*, (1990)**. The serum activity of GSH and GPx was assayed according to the kit's instructions as described by **(Ceballos-Picot *et al.*, 1992)** using spectrophotometrically at 340nm.

Histological Studies: The Ovaries of all the scarified rats were taken and immersed in 10% formalin solution. The fixed specimens will then have trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 micron thickness, and stained with Heamtoxylin and Eosin stain for examination of the ovaries as described by **Bancroft and Stevens, (1996).** The Histopathological examination was conducted at the Faculty of Veterinary Medicine, Cairo University.

Statistical analysis: The obtained results were expressed as Mean ± SD. Data was evaluated statistically by computerized SPSS package program (SPSS 22.00 software for Windows) using one-way analysis of variance (ANOVA). Significant difference among means was estimated at $p<0.05$.

RESULTS

Effect of Low Carbohydrate Diet and Anise Herb on FI, FBW, BWG and BWG (%) in Female Rats with PCOS: The results of the effect of the low carbohydrate diet (LCD) alone and the supplemented LCD with anise herb on the feed intake (FI), final body weight (FBW), body weight gain (BWG), changes in BWG (%) in female rats with PCOS are recorded in **Table 1**. The results indicated that the untreated female PCOS rats (positive control

group) fed on the basal diet (BD) alone had a significant ($p<0.05$) decrease in FI, and significant increases in FBW, BWG and BWG (%), compared to the normal control group fed on the BD. While, treating PCOS-rats by feeding on LCD results in a significant decrease ($p<0.05$) in FI, FBW, BWG and BWG (%), compared to the positive control group fed on BD. Treated PCOS-rats by feeding on the supplemented BD with anise results in significant decrease in FI, FBW, BWG and BWG %, compared to the positive control group feed on the BD alone. LCD-supplemented with anise results in a significant decrease in FI, FBW, BWG and BWG (%), compared to the positive control group.

Results also revealed that there is a significant decrease ($p<0.05$) in FBW, BWG and BWG % of PCOS rats feeding on LCD alone or the supplemented LCD with anise, compared to that fed on BD alone and the supplemented BD with anise.

Table (1): Effect of Low Carbohydrate Diet and Anise Herb on FI, FBW, BWG and BWG (%) in Female Rats with PCOS

Parameters / Groups		Parameter as Mean ± SD				
		FI (g)	**IBW (g)**	**FBW (g)**	**BWG (g)**	**Change in BWG (%)**
Negative group		13.70±0.79^{a}	203.57±2.94^{b}	245.71±3.09^{b}	42.14±2.19^{b}	20.70±3.64^{b}
Positive group		12.60±0.45^{b}	206.00±0.82^{a}	256.57±2.99^{a}	50.57±3.36^{a}	24.55±1.67^{a}
Treated PCOS rats with:	**LCD**	11.90±0.74^{c}	203.43±1.27^{b}	234.57±2.07^{c}	31.14±2.04^{c}	15.31±1.03^{c}
	BD+ Anise	11.90±0.74^{c}	204.14±1.07^{ab}	245.29±2.87^{b}	41.15±2.91^{b}	20.16±1.45^{b}
	LCD + Anise	11.90±0.74^{c}	204.85±1.35^{ab}	237.43±1.72^{d}	32.58±1.90^{c}	15.90±1.00^{c}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at $p< 0.05$. **PCOS**: Polycystic Ovary Syndrome; LCD: Low Carbohydrate Diets; **BD:** Basal Diet; **FI**: Feed Intake; **IBW**: Initial Body weight; **FBW:** Final Body Weight; **BWG:** Body Weight Gain; **%BWG:** Body Weight Gain %.

Effect of Low Carbohydrate Diet and Anise Herb on Blood glucose and Serum Insulin Levels in Female Rats with PCOS: As an evaluation of the effect of LCD, supplemented BD or LCD with anise on the levels of blood glucose and insulin hormone in female rats with PCOS, the results are recorded in **Table 2**. In

comparison to the normal rats fed on the BD, feeding PCOS rats (positive rats) on BD induced a significant ($p<0.05$) increase in serum concentrations of blood glucose and insulin hormone. On the other hand, feeding PCOS rats on the other diets (LCD and BD or LCD supplemented-with anise resulted in a significant amelioration in the serum levels of blood glucose and insulin hormone, compared with positive rats fed on BD alone. The more effectively results were observed in PCOS rats fed on the supplemented LCD with anise, compared to that of the other two diets (LCD and BD+ anise).

Table (2): Effect of Low Carbohydrate Diet and Anise Herb on Blood glucose and Serum Insulin Levels in Female Rats with PCOS

Parameters / Groups		Parameter as Mean ± SD	
		BG (mg/dl)	**Insulin (u/ml)**
Negative group		37.53 ± 1.96^{e}	0.110 ± 0.00^{c}
Positive group		73.43 ± 1.33^{a}	0.28 ± 0.19^{a}
Treated PCOS rats with:	**LCD**	60.63 ± 1.92^{b}	0.24 ± 0.14^{b}
	BD + Anise	49.87 ± 1.73^{d}	0.11 ± 0.00^{c}
	LCD + Anise	54.57 ± 1.90^{c}	0.14 ± 0.05^{d}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at $p< 0.05$. **PCOS**: Polycystic Ovary Syndrome; **LCD**: Low Carbohydrate Diets; **BD:** Basal Diet; **BG:** Blood Glucose

Effect of Low Carbohydrate Diet and Anise Herb on Serum Levels of TC, TG, TL, HDL-c, LDL-c and VLDL-c in Female Rats with PCOS: Results in Table 3 exhibit the effect of feeding PCOS rat-groups on the LCD alone and LCD or BD supplemented with anise on the serum levels of total cholesterol (TC), triglycerides (TG), total lipids (TL), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c) and very low density lipoprotein cholesterol (VLDL-c). In comparison to the negative control group fed on BD, PCOS in rats that fed on the BD induced a significant ($P < 0.05$) increase in serum concentrations of TC, TG, TL, LDL-c and VLDL-c, and decreased serum HDL-c. However, in comparison to the positive control group, feeding PCOS rats on the LCD alone and BD or LCD-supplemented with anise resulted in

significantly ($P < 0.05$) lower in the serum levels of TC, TG, TL, LDL-c and VLDL-c, and raise in serum HDL-c. A better improvement in serum levels of lipid profiles was discovered at feeding PCOS rats either on LCD alone or with LCD-supplemented with anise.

Table (3): Effect of Low Carbohydrate Diet and Anise Herb on Serum Levels of TC, TG, TL, LDL-c, HDL-c and VLDL-c in Female Rats with PCOS

Parameters / Groups		TC (mg/dl)	TG (mg/dl)	TL (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)
Negative group		126.14±1.57^{e}	101.00±1.41^{e}	481.42±1.39^{e}	63.14±1.35^{e}	42.80±1.67^{a}	20.20±2.82^{d}
Positive group		266.57±1.71^{a}	246.14±1.06^{a}	756.57±3.55^{a}	187.06±0.55^{a}	31.57±1.51^{c}	47.23±3.33^{a}
PCO Treated rats with:	**LCD**	156.14±1.57^{c}	147.28±1.60^{c}	674.00±1.91^{c}	77.32±1.18^{c}	35.42±1.13^{c}	43.40±0.38^{b}
	BD+ Anise	201.20±1.49^{b}	217.42±1.98^{b}	699.50±2.50^{b}	133.75±1.67^{b}	38.00±1.15^{b}	29.46±3.20^{c}
	LCD+ Anise	134.40±2.40^{d}	108.80±1.34^{d}	583.80±2.90^{d}	73.70±1.40^{d}	39.00±3.65^{b}	21.76±2.69^{d}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at $p< 0.05$. **PCOS**: Polycystic Ovary Syndrome; **LCD**: Low Carbohydrate Diets; **BD:** Basal Diet; **TL**: Total Lipid; **TC:** Total Cholesterol; **TG:** Triglycerides; **LDL**: Low Density Lipoprotein; **HDL**: High Density Lipoprotein; **VLDL-C:** Very Low-Density Lipoprotein Cholesterol**.**

Effect of Low Carbohydrate Diet and Anise Herb on Serum Levels of LH, FSH and testosterone in Female Rats with PCOS: Results in **Table 4** exhibit the effect of feeding PCOS rat-groups on the LCD alone and LCD or supplemented BD with anise on serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (TS). In comparison to the negative control group fed on BD, PCOS in rats that fed on the BD induced a significant ($P < 0.05$) increase in serum concentrations of LH and TS and decreased serum FSH , However, in comparison to the positive control group, feeding PCOS rats on the LCD alone and BD or LCD-supplemented with anise resulted in significantly ($P < 0.05$) lower in the serum levels of LH and TS and raise in serum FSH . A

better improvement in serum levels of hormones was discovered at feeding PCOS rats either on LCD alone or with supplemented LCD with anise.

Table (4): Effect of Low Carbohydrate Diet and Anise Herb on Serum Levels of LH, FSH and testosterone in Female Rats with PCOS:

Parameters / Groups		Parameter as Mean ± SD		
		LH (lu/ml)	FSH (lu/ml)	TS (mg/ml)
Negative group		4.77±2.13^{c}	6.94±2.29^{a}	3.07±0.96^{d}
Positive group		6.53±2.80^{a}	4.91±5.64^{d}	15.90±0.24^{a}
Treated PCOS rats with:	**LCD**	5.53±4.62^{b}	5.74±3.99^{c}	5.57±0.63^{b}
	BD + Anise	4.90±5.77^{c}	6.43±2.96^{b}	4.02±0.39^{c}
	LCD + Anise	4.55±4.69^{c}	6.95±2.75^{a}	3.07±0.54^{d}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **PCOS**: Polycystic Ovary Syndrome; **LCD**: Low Carbohydrate Diets; **BD:** Basal Diet; **LH:** Luteinizing Hormone; **FSH:** Follicle Stimulating Hormone; **TS**: Total Testosterone.

Effect of Low Carbohydrate Diet and Anise Herb on Serum Levels of E2, Progesterone, DHEA-S and Prolactin Hormones in Female Rats with PCOS: Results in **Table 5** exhibit the effect of feeding PCOS rat-groups on the LCD alone and LCD or BD-supplemented with anise on serum levels Estradiol (E2), progesterone, Dhydroepiandrosterone Sulfate (DHEA-S)**,** and prolactin. In comparison to the negative control group fed on BD, PCOS in rats that fed on the BD induced a significant ($P < 0.05$) increase in serum concentrations of DHEA-S and prolactin levels and decreased serum E2 and progesterone However, in comparison to the positive control group, feeding PCOS rats on the LCD alone and BD or LCD-supplemented with anise resulted in significantly ($P < 0.05$) lower in the serum levels of DHEA-S and prolactin and raise in serum E2 and progesterone. A better improvement in serum levels of hormones was discovered at feeding PCOS rats either on LCD alone or with LCD-supplemented with anise.

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Table (5): Effect of Low Carbohydrate Diet and Anise Herb on Serum Hormones Levels of E2, Progesterone, DHEA-S and Prolactin in Female Rats with PCOS.

Parameters / Groups		Parameter as Mean ± SD			
		E2 (ng/ml)	**Progesterone (ng/ml)**	**DHEA-S (ng/dl)**	**Prolactin (ng/ml)**
Negative group		55.39±1.48^{a}	14.19±1.98^{a}	1.39 ± 0.23^{c}	29.28±2.63^{d}
Positive group		28.86±2.62^{d}	4.22±1.57^{d}	3.93 ± 0.64^{a}	33.86±2.03^{a}
Treated PCOS rats with:	**LCD**	47.26±1.71^{c}	12.98±1.63^{c}	1.95 ± 1.02^{b}	31.71±1.98^{b}
	BD + Anise	50.61±1.91^{b}	10.47±2.73^{e}	1.95 ± 0.04^{b}	31.43±1.62^{b}
	LCD + Anise	55.38±1.46^{a}	13.01±1.60^{b}	1.03 ± 0.23^{d}	30.43±1.62^{c}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **PCOS**: Polycystic Ovary Syndrome; **LCD**: Low Carbohydrate Diets; **BD:** Basal Diet; **E2:** Estradiol; **DHEA-S:** Dhydroepiandrosterone Sulfate.

Effect of Low Carbohydrate Diet and Anise Herb on Serum Levels of MDA and Activities of Antioxidant Enzymes in Female Rats with PCOS: Results in **Table** 6 exhibit the effect of feeding PCOS rat-groups on the LCD alone and LCD or BD-supplemented with anise on the serum levels of Malondialdehyde (MDA), Catalase (CAT)**,** Superoxide Dismutase (SOD), Reduced Glutathione (GSH) and Glutathione Peroxidase (GPX) In comparison to the negative control group fed on BD, PCOS in rats that fed on the BD induced a significant ($P < 0.05$) decrease in serum concentrations of CAT,SOD,GSH and GPX and increased in serum MDA. However, in comparison to the positive control group, feeding PCOS rats on the LCD alone and BD or LCD-supplemented with anise resulted in significantly ($P < 0.05$) raise in the serum levels of CAT, SOD, GSH, and GPX, and lower in serum MDA. A better improvement in serum levels of enzymes was discovered at

feeding PCOS rats either on LCD alone or with LCD-supplemented with anise.

Table (6): Effect Serum Levels of MDA and Activities of CAT, SOD, GSH and GPx in Female Rats with PCOS of Low Carbohydrate Diet and Anise Herb on:

Parameters / Groups		Parameter as Mean ± SD				
		MDA mmol/ml	CAT (U/l)	SOD (U/l)	GSH (U/l)	GPx (U/l)
Negative group		1.89±0.215^{e}	87.14±2.67^{a}	1000.4±1.98^{a}	53.92±0.15^{a}	69.14±2.91^{a}
Positive group		3.94±.091^{a}	38.85±1.46^{d}	474.4±2.57^{e}	42.32±0.16^{e}	27.14±1.34^{e}
Treated PCOS rats with:	LCD	3.43±.100^{b}	69.00±1.63^{c}	894.1±1.86^{d}	45.03±0.13^{c}	32.20±1.49^{d}
	BD + Anise	3.23±.131^{c}	73.28±2.42^{b}	920.1±2.54^{c}	47.53±0.04^{c}	43.70±2.56^{c}
	LCD +Anise	2.53±.262^{d}	85.42±2.43^{a}	965.7±0.755^{b}	49.14±0.06^{b}	64.40±2.57^{b}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **PCOS**: Polycystic Ovary Syndrome; **LCD**: Low Carbohydrate Diets; **BD:** Basal Diet; **MAD:** Malondialdehyde**; CAT:** Catalase**; SOD:** Superoxide Dismutase**; GSH:** Reduced Glutathione**; GPX:** Glutathione Peroxidase**.**

Histopathology Examination of the Ovary: As shown in **photo 1 and 2**, the microscopic examination of ovaries from the healthy control rats revealed the normal histological structure of the ovaries including numerous corpora lutea and growing follicles in different stages. On the contrary, the examined ovaries sections from the untreated PCOS rats (positive group) exhibited numerous large thin-walled cysts (**Photo 3**), as well as, the cysts were lined by flattened epithelium and contained homogenous eosinophilic fluid (**Photo 4**). While, the mild improvement was noticed PCOS rats fed on the LCD as some of the examined sections revealed the presence of some small sized cysts (**Photo 5**) and low cuboidal to flattened lining of the cysts (**Photo 6**), meanwhile, some other sections were apparently normal (**Photo 7**). As well, marked improvement was noticed PCOS rats fed on the supplemented BD with anise, where,

the examined ovaries sections showed apparently normal follicles (**Photo 8**), and corpora lutea with some blood vessels in some instances (**Photo 9**). Likewise, LCD-supplemented with anise exhibited apparently normal ovaries (**Photo 10**)

Photo (1) Photomicrograph of ovary from normal rats showing normal structure of ovary (H&E) .

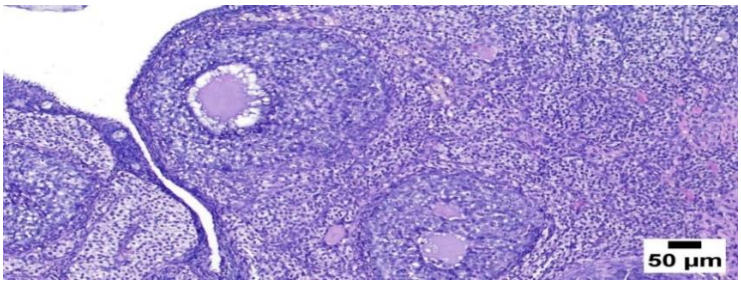

Photo (2) Photomicrograph of ovary from normal rats showing normal growing follicles and corpus luteum (H&E).

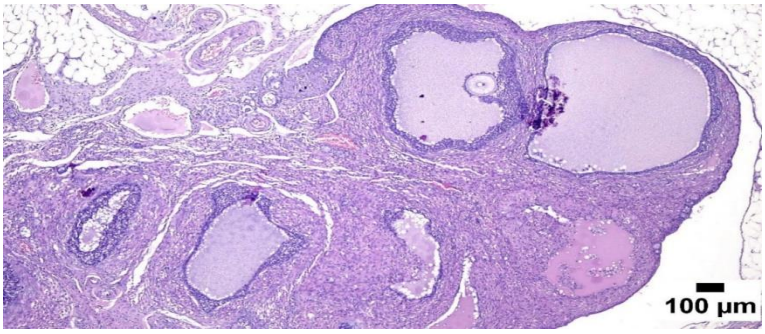

Photo (3): Photomicrograph of ovary from untreated PCOS rats (positive control group) showing numerous cystic structures (H&E)

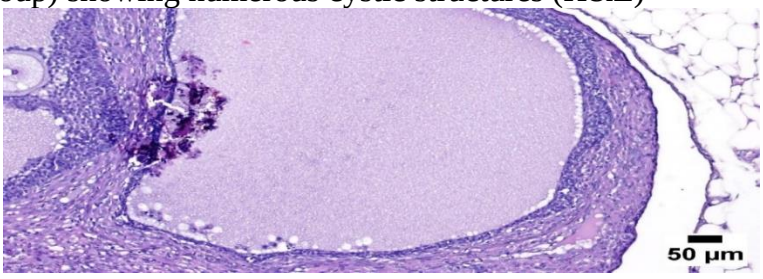

Photo (4): Photomicrograph of ovary from untreated PCOS rats (positive control group) showing thin flattened epithelial lining of the cyst (arrow) with eosinophilic homogenous fluid inside (H&E).

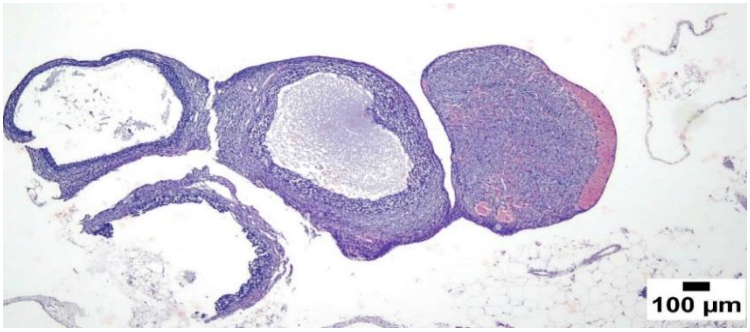

Photo (5): Photomicrograph of ovary from PCOS rats fed on the LCD showing some small cysts with apparently normal corpus luteum (H&E).

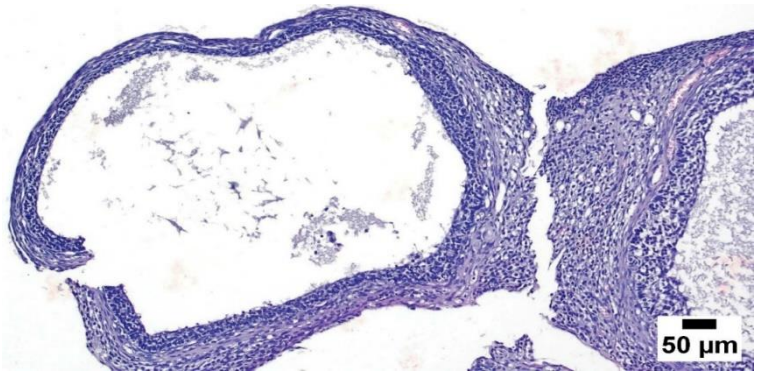

Photo (6): Photomicrograph of ovary from PCOS rats fed on the LCD showing low cuboidal to flattened lining of the cysts (H&E).

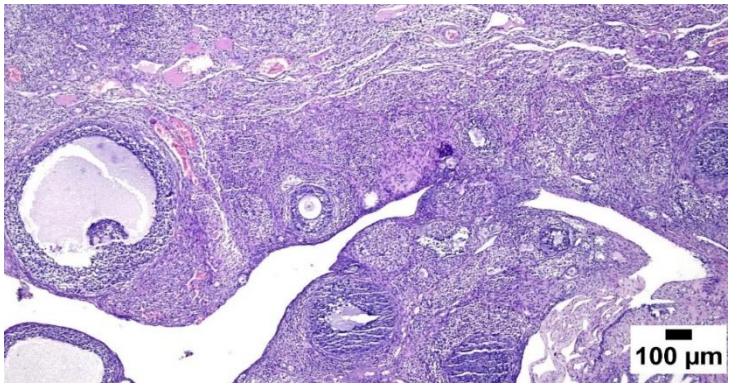

Photo (7): Photomicrograph of ovary from PCOS rats fed on the LCD showing apparently normal ovary (H&E).

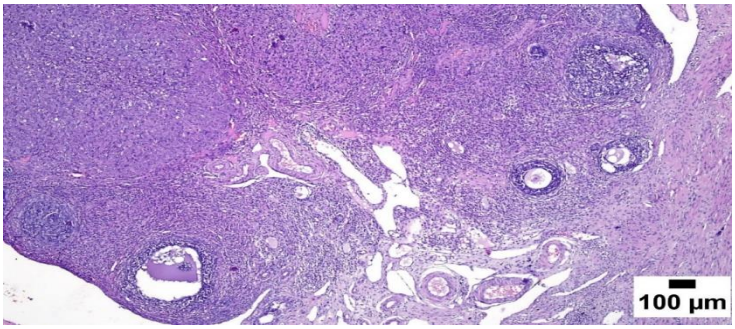

Photo (8): Photomicrograph of ovary from PCOS rats fed on the BD-supplemented with anise showing apparently normal ovary (H&E).

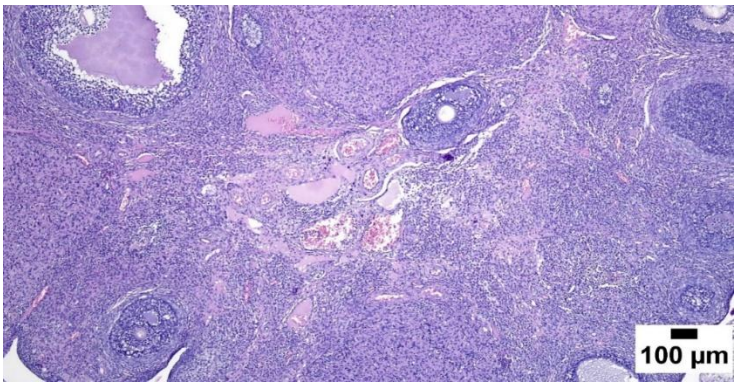

Photo (9): Photomicrograph of ovary from PCOS rats fed on the BD-supplemented with anise showing apparently normal ovary with blood vessels ectasia (H&E).

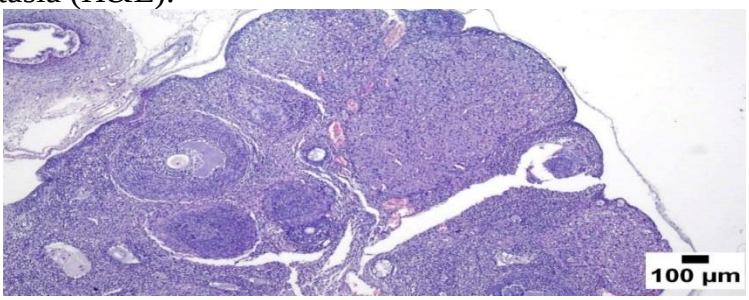

Photo (10): Photomicrograph of ovary from PCOS rats fed on the LCD-supplemented with anise showing apparently normal follicles and corpora lutea (H&E)

DISCUSSION

Polycystic ovary syndrome (PCOS) is a group of symptoms related to an unbalance of hormones that can affect women and girls of reproductive age. It is determined and diagnosed by a composite of signs and symptoms of androgen excess, ovarian dysfunction and polycystic ovarian morphology ultrasound (**Trikudanathan, 2015**).

Women from all regions of the world across the lifespan are at risk of the creature affected by PCOS. Women who are suffering from PCOS are at risk for chronic disease advancement, which stand to significant public health involvement across the longevity (**Boyle *et al*., 2012**). The metabolic abnormalities generated by PCOS, particularly increased abdominal fat and insulin resistance, bring extensive to heightened risk of type 2 diabetes and cardiovascular disease (**Ovalle and Azziz, 2002**), anxiety and depression linked with infertility, and hirsutism (**Veltman-Verhulst *et al*., 2012**). The four main risk factors for evolving PCOS are genetics, diet, and lifestyle and environmental exposes to endocrine-disrupting chemicals (**Uribarri *et al*., 2010**). Diet has been found to be a played a maior factor for PCOS, where fats and proteins diet can create advanced glycation end products (AGEs) when susceptible to sugar in the bloodstream, which provide to increased bodily stress and inflammation (**Diamanti-Kandarakis *et al*., 2012**). That effect has been audited by exploring the effect of low carbohydrate diet and anise herb on some biological and biochemical parameters, ovary histopathological in PCOS-female rats.

In current study, the obtained data revealed a significant decline ($p<0.05$) in feed intake and significant increases in body weight (BW) and weight gain (BWG) of untreated PCOS-rats fed basal diet (BD), compared to that of the healthy control rats fed BD. Additionally, the results showed a significant increase in the levels of blood glucose, and serum levels of insulin, lipid profile (TC, TG, TL, LDL-c, and VLDL-c), hormones (LH, TS, DHEA-S and prolactin) and oxidative stress as indicated by MDA. While, There is a significant decrease in serum levels of HDL-c, FSH, E2, progesterone, and activity of antioxidant enzymes (CAT, SOD, GSH and GPx), compared to that of the healthy control rats. Microscopic examination of ovaries from positive rats exhibited numerous large

thin-walled cysts, as well as, the cysts were lined by flattened epithelium and contained homogenous eosinophilic fluid.

Letrazole (LTZ) is an aromatase enzyme suppressant and associated with metabolic and reproductive disturbance (**Bhatnagar, 2007**). It can efficiently determine PCOS in rats, which develop several characteristics of human PCOS (**Kafali *et al.*, 2004**). According to prior report by **Torres *et al.*, (2019) and Negm and Aboraya, (2023)** afterwards 21 days of LTZ administration, ovarian features of PCOS can be discovered. LTZ exhibits drawbacks such as moderate weight gain and abdominal adiposity and raised fasting blood glucose. Moreover **Jena *et al.*, (2018)** founded that the central accumulation of adipose tissue was established to be characteristic of both normal weight, overweight and obese women with PCOS. Additionally, meta-analysis of thirty-five studies proved that PCOS women had a raised prevalence of central obesity, compared to women without PCOS (**Lim *et al.*, 2012**). Insulin resistance is a reduced sensitivity to the metabolic actions of insulin and one of the most essential features of PCOS (**Amisi, 2022**).

LTZ-mediated aromatase inhibition decline the transformation of androgen to oestrogen, driving to an accumulation of androgens in the ovary consequent increases testosterone and lower estrogen production (**Ndeingang *et al.*, 2019**). Lower estrogen level diminishes the negative feedback on LH production in the pituitary, resulting in increased LH levels, which furthermore encourage ovarian theca cells to secrete testosterone. This is consistent with the increased serum testosterone in Letrozole treated group (**Selim *et al.*, 2019**). Recently, Yasmine et al., (2020) exhibit marked altitude in LH and testosterone levels in comparison to control animals resulting the hyperandrogenism state in PCOS situation which the hormonal change of LTZ induced rats exhibits a hyper-androgenized state responsible for disrupted ovarian physiology. **Çınar and Gün Eryılmaz, (2016)** demonstrated the PCOS is a complex metabolic and endocrine disturbance affected both animals and women in their reproductive stage and increased the risk of reproductive irregularity. The PCOS characteristics are increased body weight, hyperglycemia, hyperinsulimia, dyslipidemia, hyper-androgenism, an-ovulation, increased level of LH and decreased level of FSH. As well, the development of the small antral follicles

and the establishment of multiple cystic ovaries were also observed in PCOS patients. Also **Fatma *et al*., (2019)** advised that LTZ administration significantly augmented the incidence of apoptosis in ovarian tissues and affected the related protein manifestation as it increased Bax expression and Bax/Bcl2 ratio however it decreased Bcl2 expression in ovarian tissue compared with normal control group. Hence, cell apoptosis has a critical role in follicular development, oocyte degeneration, follicle selection and follicle atresia which could be due to dysregulation of FSH-granular cell axis with rise of androgen level leading to deterioration of follicle selection and collection of small follicles to form cysts inside the ovary.

The previous studies demonstrated elevated levels of OS and reduced antioxidant capacity in women with PCOS (**Sabuncu *et al*., 2001**). This finding was in particular applicable in women with obesity-associated PCOS phenotype, hyperandrogenism or the establishment of metabolic symptom (**Siti *et al*., 2015**). In addition, an in vitro study exhibit that OS augmented the activity of ovarian steroid-producing enzymes and stimulated androgen production (**Piotrowski *et al*., 2005**). **Sadoughi *et al*., (2017)** stated that the production of reactive oxygen species (ROS) is considered as one of the accounting mechanisms behind infertility disturbance which results in the situation called oxidative stress. Mitochondria is the energy-producing organelles and the major source of ROS. In PCOS, mitochondrial dysfunction lead to an increased ROS production (**Yang *et al*., 2019**). Oxidative stress (OS) plays a key role in the pathophysiology of a variety of gynecological disorders, including polycystic ovary syndrome (PCOS), endometriosis, unexplained infertility, and preeclampsia (**Pizzino *et al*., 2017**). **Gao *et al*., (2023)** and **Negm and Aboraya, (2023)** noted that some women with PCOS have decreased antioxidant defense mechanisms, which can lead to an unbalance between ROS production and antioxidant defense, resulting in increased OS. In addition, an imbalance in luteinizing hormone (LH), follicle-stimulating hormone (FSH), and other reproductive hormones can affect ovarian function and may contribute to ROS production (**Ramya *et al*., 2023**). Injure to cellular structures, as a result of PUFA-induced OS, leads to the aggregation of lipid peroxidation products such as MDA and the hydroxyl radical. This enhanced OS

often results in augmented levels of ROS in the bloodstream (**Lubrano *et al*., 2019**). Additionally, there is a lowering in the activity of SOD enzyme accountable for suppressive OS. These alterations may serve to counteract the elevated OS related to PCOS and help maintain cellular integrity and function (**Enechukwu *et al*., 2019).**

With regard to the effect of low-carbohydrate diet (LCD) on PCOS-rats. The obtained results founded that LCD significantly decrease in FI, BW and BWG, and serum levels of BG, insulin, lipid profile (TC, TG, TL, LDL-c and VLDL-c), hormones (LH, TS, DHEA-S and prolactin) and MDA, while increased serum levels of HDL-c, hormones (FSH, E2 and progesterone) and activities of the antioxidant enzymes (CAT, SOD , GSH , and GPX). A low-carbohydrate diet (LCD) mention to a dietary component that supports to manage or prevent disease by restricting the intake of carbohydrates and therefore increasing the intake of proteins and/or lipids (**Meng *et al*., 2017**). It has been documented to effectively decrease body weight and promote on the treatment of infertility in obese PCOS patients (**Goss *et al*., 2014**). As well, LCD was successfully in controlling body weight in overweight or obese people, lower insulin levels, and improve insulin resistance and other endocrine deficiencies (**de Luis *et al*., 2015**). Additionally, **Zhang *et al*., (2019**) mentioned that LCD intervention caused a significant reduced in BMI, serum levels of LH and TS hormone; TC and LDL-C with increasing HDL-c and FSH hormones, compared to control diet in PCOS patients. Therefore, LCD appears to be successful as an assistant treatment for PCOS-related manifestations. Therefore, the available evidence supports the notion that LCD can effectively control body weight in overweight and obese people, lower insulin levels, and improve insulin resistance and other endocrine system deficiencies (**Wylie-Rosett *et al*., 2013**). The possible mechanism of these beneficial actions involves an LCD-induced decrease in the levels of distributed insulin and glucose (**de Luis et al., 2015**).

LCD treatment reducing the levels of glucose and insulin may have helpful effects on ovarian function. Given the principal importance of insulin receptor and compensatory hyperinsulinemia in the induction of androgen excess in PCOS women, LCD can also improve hyperandrogenism-related evidence (**Faghfoori *et al*.,**

2017). In addition, weight loss is associated with a decline in adipose tissue and therefore may negatively modulate the conversion of androgens in estrone. By this action, LCD may reduce the hypothalamic and hypophyseal dysfunction, which inspire the subfertility in PCOS women (**Zhang *et al*., 2019**). Therefore, long-term of LCD allow to restore, at least in part, insulin sensitivity in PCOS patients, counterbalance glucose metabolism disability, gonadotropin unbalance, and ovarian malfunction. Other of the possible mechanisms by which LCD improves these disturbances involves the regulation of inositol metabolism. Inositol is one of the nine stereoisomers of cyclohexanol and form part of the vitamin B family. The nearly common of cyclohexanol isomers are myoinositol and D-chiro-inositol. The wide majority of intracellular pools of myoinositol are transformed into D-chiro-inositol by an NAD/NADH-dependent isomerase, which is inhibited in PCOS due to insulin dysfunction. The unbalance between myoinositol and D-chiro-inositol plays a vital role in insulin resistance, due to the defective transformation of myoinositol into D-chiro-inositol. Therefore, the dysregulation of inositol metabolism may lead to diminished insulin sensitivity, hyperinsulinemia, inhibition of the maturation of follicles, and the development of PCOS. While with restoring the balance of inositol, there is lowering the level of androgen, and restoring the regularity of the menstrual cycle and quality of oocytes in patients with PCOS (**Lagan`a *et al*., 2018**). According to **Mahood, (2012**) who proven that lowered signs of PCOS in female rats by effects on the histomorphologies of the ovarian tissue, ameliorated the hormonal profile of PCOS.

Regarding to the effect of anise herb on the improvement of PCOS in female rats. The current study indicated that the incorporated anise herb with BS significantly improved in all of the tested biochemical parameters and ovary histopathological investigation. Aniseed contains several compounds, for example, essential oil (mainly trans-anethole), fatty acids, carbohydrates, protein, and notable amounts of phenolic compounds, such as flavonoids and phenolic acids (**Rebey *et al*., 2019**). Previous studie has indicated the pharmacological characteristic of anise fruits, including hypolipidemic, anti-diabetic, antioxidant, hepatoprotective, anti-inflammatory and dysmenorrhea-relieving (**Dadkhah *et al*., 2024**). Additionally, Due to estrogenic effects of aniseed, it was applied in

the treatment of PCOS. The randomized clinical on the efficacy of aniseed in women suffering from PCOS with complaint of oligomenorrhea indicated that anise significant augmented FSH and diminished LH, LH/FSH, testosterone serum levels after three months of treatment (**Jazani *et al*., 2018**). As Well, aniseed essential oil significantly reduced the LH and testosterone level in PCOS-animals (**Mahood, 2012**). Also, **Negahdari *et al*., (2022)** reported that one month treatment of PCOS-animals with anethole (one of the main component of anise oil) significantly reduced the insulin and MDA levels and increased SOD and CAT, as well, decreased the number of follicles, compared to the PCOS animals. The antioxidant and protective effects of trans-anethole in PCOS patients have been attributed to estrogen and phytoestrogen compounds (**Salehi *et al*., 2017**).

The free radicals and oxidative stress perform a significant role in PCOS; the production of ROS in follicles destroys the oocytes, and is the motive for oligomenorrhea and menstrual malformation (**Liu and Zhang, 2012**). Thus, the antioxidants can hinder the ROS production in ovaries and thus enhanced the ovarian functions. The present results agreed with the results of **Negm and Aboraya, (2023)** and **Dadkhah *et al*., (2024)** who mentioned that treatment of PCOS with anise herb resulted in a significant decrease MDA levels while increasing SOD and GPx activity in ovarian Furthermore, the number of follicular cysts in the PCOS rat model was significantly reduced. Recent research has stated that anise exhibits estrogenic effects, encouraging the development of ovarian follicles, restoring ovarian anatomy, lowering menopausal hot flashes, and modifying levels of LH hormone (**Mushtaq *et al*., 2023**). The action mechanism of the effects of aniseed on PCOS appeared to be related to its high content of antioxidant polyphenolic compounds such as lignans and flavonoids, which would influence oxidative stress (**Der-Marderosian and Beutler, 2002**) and estrogenic effects, which improve the menstrual cycles **Amer *et al*., (2019)**. Furthermore, dose-dependent alteration in antioxidant activity and lipid peroxidation in PCOS rats may be attributed to the effect of this extract on the oxidative genetic machinery (**Yu *et al*., 2022**).

CONCLUSION

Regard the beneficial health effect of low carbohydrate diet and the anise herb, which exhibited the ability to regulate the hormonal imbalance of polycystic ovary syndrome. The present study concluded that LCD and anise have the ability to enhance the treatment of PCOS deterioration build it a hopeful natural diet and herb for the management of PCOS. However, more investigation is required to thoroughly understand the mechanisms of action and capability benefits of LCD and anise in PCOS management.

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الملخص العربي

دراسة فعالية النظام الغذائي منخفض الكربوهيدرات وعشبة اليانسون على الفئران الإناث المصابة بمتلازمة تكيس المبايض

[١] عمرو عبدالمرضي لرزق – [٢] دينا حلمي محمد – [١] الاء اسامة ابو رية

[١] قسم التغذية وعلوم الاطعمة – كلية الاقتصاد المنزلي – جامعة حلوان
٢- قسم الهستوباثولوجي - كلية الطب جامعة القاهرة

تعد متلازمة تكيس المبايض واحدة من الاضطرابات الهرمونية الأكثر شيوعًا بين النساء في سن الإنجاب. لذلك أجريت الدراسة الحالية لمعرفة تأثير النظام الغذائي المنخفض الكربوهيدرات وعشبة اليانسون على متلازمة تكيس المبايض في إناث الفئران و الناتجة عن تناول عقار ليتروزول (١ ملجم/كجم) لمدة ٢١ يوماً. كشفت النتائج أن هناك انخفاضًا ملحوظًا في وزن الجسم النهائى ووزن الجسم المكتسب والنسبه المئوية في وزن الجسم المكتسب في الفئران المصابة بمتلازمة تكيس المبايض التي تتغذى على النظام الغذائى منخفض الكربوهيدرات وحده أوالنظام الغذائى منخفض الكربوهيدرات المكمل باليانسون، مقارنة بتلك التي تتغذى على النظام الغذائى الاساسي وحده النظام الغذائى الاساسي المكمل مع اليانسون. بالمقارنة مع مجموعة التحكم الإيجابية، فإن الفئران التي تتغذى على النظام الغذائى منخفض الكربوهيدرات وحده و النظام الغذائى الاساسى أو النظام الغذائى منخفض الكربوهيدرات المكملين باليانسون لديها تحسن كبير في مستويات جلوكوز في الدم، الكوليستيرول الكلى، والدهون الثلاثيه، الدهون الكلية، الليبوبروتينات المنخفضة والمنخفضة جداً والمرتفعة الكثافة، بالإضافة إلى هرمون الأنسولين، اللوتين، المحفز للحويصلات، التيستيستيرون، استراديول، البروجسترون، هيدروإيبي أندروستيرون وهرمونات البرولاكتين، ونشاط الإنزيمات المضادة للأكسدة. تم اكتشاف أفضل تحسن في مستويات السيرم من المؤشرات المذكورة أعلاه والفحص الهستوباثولوجي للمبيض عند الفئران المصابة بمتلازمة تكيس المبايض والتي تغذت على النظام الغذائى المنخفض الكربوهيدرات وحده أوالنظام الغذائى منخفض الكربوهيدرات والمكمل باليانسون، مقارنة بالمجموعات الأخرى. أخيرًا، فإن قدرة اليانسون والنظام الغذائى منخفض الكربوهيدرات على تعزيز علاج متلازمة تكيس المبايض تجعله نظامًا غذائيًا طبيعيًا وعشبًا للتحكم في مشاكل متلازمة تكيس المبايض.

الكلمات المفتاحية: الهرمونات الجنسية، الملف الدهني، متلازمة نكيس المبايض، نظام غذائي منخفض الكربوهيدرات