Egypt. J. Vet. Sci. **Vol. 56,** No. 2, pp. 257-271 (2025)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

The Role of Oxidative Stress on Cardiovascular Properties of the Androgen- Induced PCOS in Female Rats

Sukayna H. Rashed*, Saba Z. Al-Abachi and Raghad A. M. Hamoo
Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq

HUMAN and animal anovulation is a result of polysystolic ovary PCOS which involves endocrine, reproductive, and metabolic abnormalities. Finding new medications with least amount of toxicity is a major scientific challenge. The study's goal is to assess biochemical variables in blood serum and heart tissue in female rats as models with androgen-induced PCOS and the impact of oxidative stress in development of cardiovascular characteristics resulting from PCOS. PCOS-induced rats were treated with liquorice root [L.], green tea [GT], and marjoram [M.] extracts at 150, 250 and 100 mg Kg^{-1} B.W. respectively. Metformin [Met.] 20 mg kg^{-1} B.W. used as a standard medication. The study employed 36 female rats aged 8-10 weeks. Six [n=6] groups of rats were formed. At zero time and at the end of the trial, blood was obtained from all rats to estimate total cholesterol, triglycerides, HDL-C, LDL-C, VLDL-C, glucose, total protein, CRP, glutathione, malondialdehyde, glutathione peroxidase, AST, ALT, ALP, testosterone, estradiol, progesterone in blood and CK, LDH, IL-1β and TNF-α in heart tissue. In female rats treated with androgen, there has been a rise in all measured parameters except HDL-C, estradiol, progesterone, GSH, GSH-Px and, which increased again in three extracts-treated groups. M. extract was more active than GT and L. extracts in improvement of cardiovascular [CV] parameters. Since CK, LDH and IL-1β were regained normal values. In conclusion, study demonstrated that M., GT, and L. can decrease the negative effects of PCOS in particular on cardiovascular features, and treating PCOS with these herbs is recommended.

Keywords: Androgen, Green tea, Liquorice root, Marjoram, Polycystic ovary syndrome.

Introduction

Polycystic ovarian syndrome, also known as PCOS constitutes one of the more prevalent primary hormonal disorders, especially impacting women of older age for reproduction and the main cause emerges as a combination of, clinical, hormonal and anatomical indications, anovulatory infertility, irregular menstrual cycles, increased testosterone concentrations [hyperandrogenism], the blood serum of hormonal disorders as hyperinsulinemia, acne, obesity, reduced fertility and hair loss [1,2]. According to studies, various endocrine problems in PCOS reinforce and intensify one another. These illnesses include impairments the hypothalamic-pituitary pathway functioning, ovarian and adrenal function, and hormonal problems [3]. In reality, PCOS is linked to reproduction, metabolic, psychological dysfunction, and clinical complications examples include reduced tolerance for glucose and diabetes, hypertension, severe cardiovascular disease, chronic oligo-ovulation, infertility, anovulation, and ovarian cancer. Despite a lengthy history of research on PCOS, the aetiology is yet unclear, but have revealed involvement in an inflammatory state, endothelial injury, oxidative stress [OS], in addition genetic pathways [4,5].

Many studies indicate the oxidant circulation

*Corresponding author: Sukayna H. Rashed, E-mail: sukaynarashed@uomosul.edu.iq, Tel:009647740942674
(Received 20/02/2024, accepted 17/04/2024)
DOI: 10.21608/EJVS.2024.271704.1865
©2025 National Information and Documentation Center (NIDOC)

markers are much greater in women with PCOS than in the general population, and they are now recognized as a possible cause of PCOS aetiology. It is already established as playing an important role in the pathophysiology of a variety diseases, including PCOS. It has gained substantial interest in recent twenty years since the discovery that inappropriate oxidation state was associated to individuals with chronic illnesses, such as diabetes, cardiovascular, PCOS, neurological disorders and cancer [6-7]. In the setting of PCOS, numerous variables may increse reactive oxygen species [ROS] production or decrease antioxidant defence. The OS is other participant component in various reproduction illnesses like endometriosis, PCOS, or unresolved infertility caused the discovery that inappropriate oxidants and antioxidants [8].

The underpinning processes of PCOS are complex and diverse, with hormone abnormalities playing a critical role. The ovaries generate high levels of androgens, particularly testosterone. The elevated androgen concentrations result from abnormal insulin metabolism, since a considerable majority of PCOS-affected women exhibit insulin resistance, resulting in reactive hyperinsulinemia. This insulin spike, in unison with luteinizing hormone [LH], stimulates androgen production in the ovaries [9-10].

The testosterone enanthate, a synthetic steroid with androgenic qualities, has been linked to PCOS. Despite OS and inflammation are the two primary routes implicated in the aetiology of PCOS [11].

Various treatments have been proposed to treat this syndrome, including changes in lifestyles, surgery, also the uses of drug. Current therapy options include clomiphene citrate, metformin, tamoxifen, and gonadotropins. Regarding the detrimental consequences of various therapies, the development of alternative medications includes the usage of herbal plants and their derivatives has garnered a lot of attention because it›s less intrusive, cheaper, and more useful than other ways [12].

Herbs are the oldest kind of medicine, and their therapeutic effects and applications are well recognized. A return to natural processes and the administration of plant-derived medications happens in the time when modern person has experienced the side effects and complications of chemical drugs as a result of his promotion of their usage.

Investigations in science proved the efficacy and safety of numerous alternative healthcare therapies, using herbs in management of specific disorders [13].

Glycyrrhiza glabra L. roots are annual, moderate zone plant, or subshrub whose primary active component is the triterpenoid saponin glycyrrhizin, flavonoids, gums, starches, essential oils, chalcones, and volatile oils. Licorice is reported to display the following pharmacological measures: estrogenic, aldosterone-like activity, antibacterial, and antitrichomonas [14].

Green tea is produced from the unoxidized leaf of the Camellia sinensis plant. This belongs to the group of the most treated teas, with the highest concentrations of antioxidants and beneficial polyphenols. According to some research, green tea might be beneficial for weight reduction, liver illness, and POCS. For PCOS, green tea extract lowers fasting insulin and testosterone levels, which are two important aspects of this disorder. Green tea has many healthful actions, in the context of polycystic ovarian syndrome, a major benefit is improving insulin sensitivity as insulin resistance is a key factor in this hormonal condition [15].

Marjoram [Origanum majorana] is a cold-sensitive annual plant or shrub with pleasant pine and citrus flavours. It has traditionally been regarded as a medicinal herb. Marjoram, or marjoram oil, has been employed to treat colds, coughs, cramps, paralysis, arthritis, chest congestion, and muscular pain. The herb has confirmed medicinal properties. For instance, it includes phenol carvacrol that is antibacterial, antifungal, and antimicrobial. Dried marjoram, marjoram tea, or chemicals derived from marjoram have been shown to have cardioprotective, hepatoprotective, anti-PCOS, and anti-inflammatory properties [16].

Metformin, often known as Glucophage, belongs to the biguanide class of medicines. It inhibits gluconeogenesis, lowering blood sugar levels and body weight. It could be increase fibrinolysis activity, insulin sensitivity, and utilization in peripheral tissues including skeletal muscle and adipocytes. Metformin is also the best medicine for treating PCOS [17].

Therefore, we aimed to evaluate the biochemical variables in blood serum and heart of isolated rat hearts in androgen-induced PCOS form in rats, in addition to the impact of

OS in the emergence of certain cardiovascular characteristics associated with PCOS.

Material and Methods

Extracts preparation

Preparation of cold aqueous crude extract: 100 grams of licorice root powder, green tea and marjoram were weighed, then 300 milliliters of cold distilled water were added to it at a ratio of [1:3] and the mixture was crushed for 10 minutes using a blender. Then stir for a full hour and freeze mixture for 72 hours, with cracking every 24 hours to break down the cell walls. Then filtration to get a clear filtrate, where volume of the filtrate was [217, 202, [208 ml for licorice, green tea, and marjoram, respectively. Refrigerated till further analysis [18].

Animals

The study used female Wistar albino rats that were 8–10 weeks old. The animals that were inbred were obtained from animal house at University of Mosul, Mosul, Iraq's College of Veterinary Medicine. The rats were kept in propylene cages in a ventilated room, with a natural 12±1 h day/night cycle. They were given a balanced rodent diet consisting of pellets and tap water on demand.

They were housed in the lab for a week in order to acclimate before the experiment. The Scientific Committee of Chemistry Department Council at the College of Science, University of Mosul approved the experimental protocol at the 12th session in April 4/5, 2022.

Experimental Design

Six groups of six animals each were created by randomly selecting the 36 female rats: Group I: [Control group normal]. Group II acted as a negative control and was given testosterone enanthate[TE] only. Group III consisted of rats with PCOS who received a 28-day oral treatment of Glycyrrhiza glabra [L.] 150 mg kg−1. Group IV consisted of rats with PCOS who were given green tea [GT] 250 mg kg−1 orally every day for 28 days. Group V consisted of rats with PCOS who were given Marjoram [M.] 100 mg kg−1 orally every day for 28 days. Group VI: Received routine care and was administered Metformin [Met.] 20 mg kg−1 orally every day for 28 days [18].

For five weeks, PCOS was created by injecting sesame oil-dissolved testosterone enanthate [TE] at a rat of 1mg/100 g of body weight [BW] subcutaneously. For five weeks, 0.1 milliliter of sesame oil was given to the animals in the CTRL group [19-20]. BW was assessed every day of the experiment in order to determine the dosages of extracts. To minimize the influence of manipulation on animal welfare, injections were administered every day at the same time [10:00–10:30 h]. Blood samples were taken before the extracts were administered and again 28 days after the extracts were given at the start of the experiment [after the PCOS was developed], serum blood samples were kept for later examination at [-20°C] whereas the CRP estimation was made right away. Animals were given intraperitoneal injections of xylazine [5 mg/kg] and ketamine [10 mg/kg] to induce anesthesia twenty-four hours following the last TE injection. They were then beheaded for sacrifice, hearts were extracted from their chest cavities, and organized for the biochemical variables to be measured.

Evaluation of Estrous Cycle

The estrus cycle was tracked every day for the final two weeks of the experiment using the cytological analysis of vaginal smears. In summary, vaginal lavage was carried out every morning at 9:00 a.m. before to therapy using a dropper that was partially filled with distilled water. A light microscope was used to examine the lavage after it had been put on a glass slide and stained with hematoxylin. Predominance of particular cells allowed for the following identification of estrus cycle phases: Round, nucleated cells make up proestrus; cornified squamous cells make up estrus; leucocytes and cornified squamous cells make up metestrus; and nucleated epithelial cells and leucocytes predominate during diestrus [21].

Preparing Isolated Rat Hearts

Following a 28-day course of therapy, all hearts were measured and excised and the left ventricles were utilized for biochemical examination. After homogenizing 0.5 g of heart tissue in ten volumes of ice-cold phosphate-buffered saline [pH 7.4], the homogenate was centrifuged for 20 minutes at 4°C at 1200 ×g. Supernatants were stored at -70°C until the analyses were completed [22].

Biochemical analysis

Biochemical parameters were measured in the treated and control animals' blood serum. Using an Accu Chek glucometer, the blood serum glucose concentration was assay on first days and 28 of trial [19-23]. TC, TG, HDL-C using BioMérieux kit [France], commercially accessible kits from AMP Diagnostics AMEDA Labordiagnostik GmbH, Austria, were used to analyze total protein [18] and CRP. Every process was carried

out in accordance with the manufacturer's instructions. The Friedewald formula was used to compute the VLDL-C and LDL-C [24-25].

Spectrophotometric analysis was used to measure the amount of [GSH], which is based on GSH oxidation through 5,5-dithiobis-6,2-nitrobenzoic acid. The measurement was performed at 420 nm [23]. The MDA was determined according to Ohkawa *et al.,* [34].

Levels of glutathione peroxidase were measured in accordance with Mohandas *et al* [26].

Colorimetric determination is used to examine each sample for ALT, AST, and ALP using the BIOLAO kit [18] and bioMérieux [19].

Using commercially available ELISA kits, the levels of serum total testosterone, progesterone, and estradiol were assessed [Bioassay Technology Laboratory BT LAB, Shanghai, China]. Manual instructions were used to complete every procedure. Heart tissue analyzed in duplicates with rat IL-1β ELISA kit [cat. No. E-EL-R0012, Elabscience Bionovation, USA] with a sensitivity of 18.75pg/ ml, TNF-α ELISA kit [cat. No. E-EL-R2856, Elabscience Bionovation, USA] with a sensitivity of 9.38pg/ ml, Creatine phosphokinase activity determined using coloimetric method that CK activity calculated by measuring the OD values at 430nm [cat. No. E-BC-K558-S, Elabscience Bionovation Inc., USA] with a sensitivity of 3.7U/ L and Lactate dehydrogenase activity determined using coloimetric method with a sensitivity of 6 U/ L [cat. No. E-BC-K046-M, Elabscience Bionovation Inc., USA].

Statistical Analysis

The data were assessed using the t-test, Duncan's multiple test and values were shown as the mean ± standard deviation. Version 22.0 of the SPSS statistical software was used to conduct these analyses. A P-value of less than 0.05 was regarded as statistically significant.

Results

Administration of androgen [1 mg/100 g BW] to female rats caused there to be a notable rise in serum TC, TG, LDL, and VLDL whereas there was a significant reduction in HDL-C in comparison with POCS group. The POCS+L, POCS+GT, POCS+M and metformin groups were changed but non-significant in the initial experiment, while after 28 days the decreased were significantly in VLDL-C, TG, LDL-C, and TC, while there was a remarkable rise in HDL-C in comparison to POCS group as shown in [Table1] and [Table2]. The Origanum majorana gave highest height followed by Glycyrrhiza glabra L. then Camellia sinensis in HDL-C concentration when in contrast to the POCS group.

TABLE 1. Impact of various extracts on blood serum TC and TG concentrations on zero time and 28 days of experiment rats with PCOS

Groups n=6	TC [mg\dl]		[TG mg\dl]	
	Zero time	28 days	Zero time	28 days
G1	80.3±5.4^{A}	81.7±2.3^{a}	70.2±2.1^{A}	68.9±2.4^{a}
PCOS	89.8±6.1^{B}	93.5±5.4^{c}	91.6±5.2^{B}	95.1±6.1^{b}
PCOS+L [150 mg\Kg BW]	87.4±5.5^{B}	79.8±5.3^{a}	90.1±6.7^{B}	73.4±3.3^{a}
PCOS+GT [250 mg\Kg BW]	86.6±6.8^{B}	78.4±6.1^{a}	89.8±5.1^{B}	69.4±6.5^{a}
PCOS+M [100mg\Kg BW]	86.7±5.9^{B}	72.8±5.7^{a}	90.9±6.4^{B}	70.7±4.7^{a}
PCOS+Met [20 mg\Kg BW]	87.2±4.9^{B}	77.7±6.4^{a}	90.6±5.8^{B}	73.8±3.8^{a}

Values are Mean ± SD, Values in the same column sharing the same letters are non-significant different at $P \leq 0.05$.

TABLE 2. Impact of various extracts on blood serum HDL-C, LDL-C and VLDL-C concentrations on zero time and 28 days of experiment rats with PCOS

Groups n=6	HDL-C [mg\dl]		LDL-C [mg\dl]		VLDL-C [mg\dl]	
	Zero time	28 days	Zero time	28 days	Zero time	28 days
G1	47.7 ± 2.7^{C}	46.8 ± 3.1^{C}	28.1 ± 2.1^{a}	27.6 ± 1.3^{a}	14.4 ± 1.1^{a}	13.78 ± 1.2^{a}
PCOS	25.1 ± 2.5^{Ab}	$21.7\pm2.0^{A*}$	65.6 ± 4.8^{b}	63.8 ± 6.1^{b}	18.1 ± 1.1^{b}	19.04 ± 1.3^{b}
PCOS+L [150 mg\Kg BW]	25.4 ± 1.7^{Ab}	30.1 ± 2.2^{B}	67.5 ± 3.8^{b}	29.1 ± 2.7^{a}	18.1 ± 1.4^{b}	14.7 ± 0.6^{a}
PCOS+GT[250 mg\Kg BW]	23.7 ± 2.1^{A}	29.7 ± 1.4^{Ab}	62.4 ± 5.1^{b}	25.8 ± 1.9^{a}	17.9 ± 1.02^{b}	13.9 ± 1.3^{a}
PCOS+M[100mg\Kg BW]	21.8 ± 1.2^{A}	32.3 ± 2.5^{Bc}	63.3 ± 4.7^{b}	28.9 ± 2.4^{a}	18.2 ± 1.3^{b}	14.1 ± 0.9^{a}
PCOS+Met [20mg\Kg BW]	23.9 ± 1.9^{A}	30.3 ± 2.8^{B}	62.8 ± 4.5^{b}	30.1 ± 2.5^{a}	18.1 ± 1.2^{b}	14.8 ± 0.8^{a}

Values are Mean ± SD, Values in the same column sharing the same letters are non-significant different at $P \leq 0.05$. The symbol* refers to statistically significant at $P \leq 0.05$.

On the first day of trial blood glucose levels were determined, table 3 shows the Mean±SD. The mean values of the groups did not significantly differ following the initiation of PCOS inducing. although glucose levels in the PCOS group was significantly higher than in the control group. On day 28, when the trial came to its end, glucose levels were measured once again. just before the commencement of post therapy. Different extract groups POCS+L, POCS+GT and POCS+M depicted significant decrease when compared with PCOS group, whereas they illustrated significant increase relative to the group of control. In addition, the occurrence of androgen-induced PCOS raises blood protein levels; however, following 28 days of three extracts dosing, the percentage of blood protein concentrations was minimal [28.21%, 35.49%, 37.86%], in comparison to metformin which dropped TP to 36.88% [**Table 3**].

When compared to the control group, the PCOS group's TP concentration rose significantly, while there were significantly decrease in the POCS+L, POCS+GT and POCS+M groups in addition of metformin group after 28 days and comparing to the PCOS group.

Table 3 indicates that there were no changes in CRP data in the PCOS group compared to the other groups. When the GSH concentration was compared between the POCS group and the control group, a notable drop was seen, while there was a significant increase in POCS+L, POCS+GT, POCS+M and in Metformin groupings when compared with POCS group after 28 days.

Table 3 showed a significant rise in the MDA concentration, also, showed a significant decrease in POCS+L, POCS+GT, POCS+M and in metformin groups when compared with POCS and in control group after 28 days.

The findings on the preventive benefits of POCS+L, POCS+GT and POCS+M against PCOS in rats ovaries and of antioxidant enzyme like GSH-Px activity, was shown in Table 4. In comparison to the control group, the PCOS group had significantly less GSH-Px activity. This decrease in GSH-Px activity was not significantly reversed after 28 days of treatment with POCS+L, POCS+GT, POCS+M, and metformin.

The POCS group showed a considerable rise in AST, ALT, and ALP activities as compared to the control group, while there was a significant decrease in POCS+L, POCS+GT, POCS+M and in metformin groups when compared with POCS group after 28 days of experiment [Table 5].

Compared to the control group, PCOS female rats had a highly significant increase in serum sex hormones like testosterone. while the POCS+L, POCS+GT, POCS+M and metformin groups exhibited a significant decrease in sex hormones comparison with PCOS [group 2] after 28 days. Significant declines were observed in the values of

TABLE 3. Impact of different extracts on blood serum glucose, TP and CRP concentrations on rats with PCOS

Groups n=6	Glucose [mg\dl]		Total protein [mg\dl]		CRP [mg\dl]	
	Zero time	28 days	Zero time	28 days	Zero time	28 days
G1	76.4±2.1^{A}	73.23±3.9^{a}	27.5±1.78^{a}	25.73±1.85^{A}	0.052±0.004^{a}	0.051±0.002^{a}
PCOS	85.3±4.1^{Ab}	96.6±2.2^{b}	39.81±3.1^{b}	40.45±2.7^{B}	0.071±0.002^{a}	0.072±0.001^{a}
PCOS+L [150 mg\Kg BW]	83.21±2.4^{Ab}	81.5±5.2^{ab}	40.12±4.1^{b}	28.8±2.7^{A}	0.069±0.001^{a}	0.062±0.001^{a}
PCOS+GT [250 mg\Kg BW]	86.4±3.3^{Ab}	80.9±4.7^{a}	41.7±5.1^{b}	26.9±2.2^{A}	0.069±0.003^{a}	0.060±0.001^{a}
PCOS+M [100mg\Kg BW]	86.6±5.3^{Ab}	80.1±3.4^{a}	41.2±1.9^{b}	25.6±2.8^{A}	0.07±0.002^{a}	0.059±0.001^{a}
PCOS+Met [20mg\Kg BW]	84.9±4.4^{Ab}	74.9±1.7^{a}	40.4±2.4^{b}	26.5±3.3^{A}	0.071±0.003^{a}	0.099±0.001^{a}

The values are expressed as Mean ± SD, and at $P \leq 0.05$, values that are in the same column and have same letters differ non-significantly.

TABLE 4. Effect of different extracts on blood serum GSH, MDA concentrations and GSH-Px activity on zero time and 28 days of experiment rats with PCOS

Groups n=6	GSH [µmole\L]		MDA [µmole\L]		GSH-Px [u\mol\mg]	
	Zero time	28 days	Zero time	28 days	Zero time	28 days
G1	6.52±0.02^{d}	6.38±0.08^{d}	6.99±0.64^{a}	6.74±0.82^{a}	28.17±1.84^{B}	28.12±2.21^{b}
PCOS	3.15±0.17^{a}	3.13±0.11^{a}	14.57±0.89^{c}	15.59±1.14^{c}	19.55±1.88^{A}	20.17±1.92^{a}
PCOS+L [150mg\KgBW]	3.17±0.08^{a}	4.62±0.27^{b}	14.77±0.76^{c}	10.17±1.4^{b}	20.42±1.45^{A}	24.22±1.08^{b}
PCOS+GT [250mg\KgBW]	3.28±0.07^{a}	4.87±0.41^{b}	14.68±0.27^{c}	9.48±0.71^{b}	18.79±1.44^{A}	25.17±2.11^{b}
PCOS+M [100mg\KgBW]	3.17±0.09^{a}	5.25±0.18^{c}	15.08±0.81^{c}	8.71±0.88^{ab}	19.99±2.05^{A}	28.57±2.32^{b}
PCOS+Met [20 mg\KgBW]	3.24±0.14^{a}	4.88±0.32^{b}	14.61±0.93^{c}	9.58±0.52^{b}	20.59±2.71^{A}	25.25±1.48^{b}

Values are Mean ± SD, Values in the same column sharing the same letters are non- significant different at $P \leq 0.05$.

TABLE 5. Impact of different extracts on blood serum AST, ATT and ALP activities on zero time and 28 days of experiment rats with PCOS

Groups n=6	AST[U\L]		ALT[U\L]		ALP[U\L]	
	Zero time	28 days	Zero time	28 days	Zero time	28 days
G1	34.1±1.4^{A}	35.1±1.7^{a}	23.3±2.1^{a}	24.5±1.9^{a}	60.2±1.6^{a}	59.8±2.4^{a}
PCOS	50.1±4.2^{B}	48.7±4.6^{b}	45.7±3.1^{c}	45.5±2.3^{c}	93.4±5.3^{c}	91.1±6.3^{c}
PCOS+L [150mg\KgBW]	49.5±2.8^{B}	37.8±4.1^{a}	46.1±4.1^{c}	32.7±2.4^{b}	91.4±4.4^{c}	69.1±6.2^{b}
PCOS+GT [250 mg\KgBW]	48.5±1.9^{B}	39.4±2.7^{b}	48.7±3.2^{c}	29.2±2.7^{a}	92.7±5.1^{c}	61.8±4.8^{a}
PCOS+M [100 mg\KgBW]	47.7±4.3^{B}	42.7±2.4^{ab}	44.5±4.2^{c}	30.8±2.5^{b}	90.8±7.1^{c}	63.1±3.8^{a}
PCOS+Met [20mg\Kg BW]	46.9±3.2^{B}	38.5±3.3^{a}	44.8±3.6^{c}	30.9±2.7^{b}	90.9±4.7^{c}	61.4±5.2^{a}

Values are Mean ± SD, Values in the same column sharing the same letters are non-significant different at $P \leq 0.05$.

estradiol in the POCS group when compared with the control group, but there were no significant changes among rats of all the themselves groups at the beginning of the experiment, while, there was a significant increase in extract groups compared with PCOS group after 28 days. When compared to the control group, the PCOS group's progesterone level significantly decreased as a result of androgen treatment [Table 6], while there was a significant increase in POCS+L, POCS+GT, POCS+M and metformin groups when compared with POCS group.

Before pounding the heart tissue, its weight was determined and then compared to the weights of the healthy control group's hearts; additionally, the final body weight of rats was measured at the end of the experiment, as shown in Table 7, indicating that there were no significant differences in the PCOS and control groups in heart weight and in final body weight. Also, when compare all of the trial groups.

At the last day of the trial [28 days], the experimental animals were being killed**.** the heart was taken from each group and the following biochemical tests were performed: Tumor necrosis factor-alpha [TNF-α], lactate dehydrogenase [LDH], creatine kinase [CK], and interleukin-1 beta [IL-1β].

TABLE 6. Impact of different extracts on blood serum testosterone, estradiol, and progesterone levels on zero time and 28 days of experiment rats with PCOS

Groups n=6	Testosterone [ng\ml]		Estradiol [ng\mg]		Progesterone [pg\ml]	
	Zero time	28 days	Zero time	28 days	Zero time	28 days
G1	1.91±0.66^{A}	1.96±0.25^{a}	20.11±2.10^{a}	19.84±1.41^{a}	42.35±2.29^{c}	41.51±1.14^{c}
PCOS	10.22±0.57^{D}	10.18±0.27^{d}	14.61±0.72^{b}	13.99±0.94^{b}	13.69±4.51^{a}	14.1±1.4^{a}
PCOS+L[150mg\KgBW]	10.21±0.43^{D}	8.12±1.98^{c}	14.55±0.47^{b}	28.32±0.22^{c}	13.78±1.4^{a}	22.78±2.1^{b}
PCOS+GT[250mg\KgBW]	10.14±0.97^{D}	5.36±0.49^{b}	14.35±0.61^{b}	25.35±0.37^{c}	14.17±0.9^{a}	22.81±1.9^{b}
PCOS+M[100mg\KgBW]	9.98±0.29^{D}	5.72±0.54^{b}	14.41±0.27^{b}	30.92±6.1^{c}	13.88±1.1^{a}	21.67±0.9^{b}
PCOS+Met[20mg\KgBW]	10.14±0.63^{D}	4.55±0.29^{b}	14.15±1.1^{b}	29.77±2.7^{c}	13.72±1.4^{a}	24.37±2.2^{b}

The values in the same column with the same letters differ not significantly at P ≤ 0.05. The values are Mean ± SD.

TABLE 7. Effect of different extracts on heart weight and final body weight after 28 days of experiment rats with PCOS

Groups n=6	Heart weight [HW] [gram]	Final body weight [gram]
G1	1.04±0.01^{a}	236±2.79^{A}
PCOS	1.24±0.02^{a}	241±2.5^{A}
PCOS+L [150mg\Kg BW]	1.21±0.01^{a}	239±2.6^{A}
PCOS+GT[250mg\Kg BW]	1.10±0.02^{a}	238.5±1.5^{A}
PCOS+M[100mg\Kg BW]	1.08±0.03^{a}	239.1±2.4^{A}
PCOS+Met [20mg\Kg BW]	1.09±0.02^{a}	239.7±1.7^{A}

The values in the same column with the same letters differ not significantly at P≤0.05. The values are Mean ± SD.

TABLE 8. Impact of different extracts on CK, LDH, IL-1β, and TNF-α on heart tissue after 28 days of experiment rats with PCOS

Groups n=6	CK [U\L]	LDH [U\L]	IL-1β [pg\ml]	TNF-α [pg\ml]
G1	44.57±2.6^{a}	67.2±5.9^{A}	54.2±2.2^{a}	68±4.04^{a}
PCOS	111.7±5.2^{c}	118.0±10.4^{C}	150.4±5.3^{d}	681±16.57^{b}
PCOS+L [150mg\Kg BW]	75.8±2.5^{b}	90.4±4.4^{B}	76.8±7.3^{c}	690±20.77^{b}
PCOS+GT [250mg\Kg BW]	78.4±6.1^{b}	88.7±4.3^{B}	71.8±8.4^{c}	685±22.81^{b}
PCOS+M [100mg\KgBW]	50.1±4.2^{a}	71.2±4.6^{A}	56.4±6.3^{a}	687±31.47^{b}
PCOS+Met [20mg\Kg BW]	69.3±3.3^{b}	83.1±3.8^{B}	65.7±7.9^{b}	687±15.65^{b}

Values are Mean ± SD, Values in the same column sharing the same letters are non-significant different at $P \leq 0.05$.

In comparison to the control group, there was a substantial increase in CK activity in the PCOS group. Additionally, Table 8's LDH activity data demonstrated a statistically significant increase in the POCS group relative to the control group.

When we evaluated the amount of IL-1β in the cardiac tissue of female rats with POCS, we discovered that the affected group's level had significantly increased in comparison to the control group. According to Table 8, the PCOS group's Tumor Necrosis Factor-alpha increased significantly when compared to the control group.

The results recorded a significant decrease in IL-1β in the heart tissue of the groups treated with the extracts, where the highest decrease was in the group treated with marjoram extract, reaching 56.4pg/ml compared to the negative control group 150.4pg/ml, followed by green tea 71.8pg/ml and finally licorice roots 76.8pg/ml, which are good drop ratios compared to metformin as a standard treatment. Groups 3 to 6 showed a notable improvement in cardiac tissue activity for both CK and LDH, with the fifth group treated with marjoram showing the highest activity levels [70.1U/L&81.2U/L] for both CK and LDH, respectively, compared to the negative control group [111.7U/L&118U/L] [Table 8].

Discussion

Our results for lipid profile were corresponding with other studies such as [27-28], which they indicate that the dyslipidemia in PCOS which have multiple causes, and insulin resistance performs plays a crucial function by encouraging lipolysis and altering lipases, that change marked by greater TG and decreased HDL-C, and therefore Insulin resistance and hyperandrogenism are significant contributions to women's higher cardiovascular risk with PCOS [29]. Also, they noted that the dyslipidaemia is a returning CVD risk factor observed in women with PCOS. A previous study found that a considerable number of women with PCOS [70%] had dyslipidaemia. likewise, a meta-analysis of 30 research establishes that PCOS in women have a highly concentration of lipids, specifically HDL-C, LDL-C, and TG. Furthermore, TG and HDL-C levels were significantly higher in the obese strata, suggesting a probable relationship between PCOS and obesity. As a result, the most recent guidelines recommend that women of all ages be diagnosed and undergo a lipid profile [30].

Most research claimed that the examined herbal extracts such as Camellia sinensis and Origanum majorana were helpful in managing PCOS and improving sex hormone levels, insulin resistance, hyperandrogenism, and markers of PCOS. The Glyrrhiza Glabra L. root which contain sterols or phytoestrogens can lower TC and TG [31-32].

PCOS women are thought to have aberrant lipid profiles when compared to weight- and age-matched counterparts. The most obvious anomalies are high TG and low HDL-C, both of which are key indicators of CVD and myocardial infarction. Metformin has the potential to effect dyslipidemia directly by affecting fatty acid metabolism in the liver or indirectly by reducing hyperinsulinaemia. Numerous investigations suggest using metformin drug had a beneficial effect on dyslipidemia in PCOS women [33].

Glucose levels increased considerably in the PCOS group [96.6±2.2 mg\dl] during the day 28 of the experiment when compared with control group [73.23±3.9 mg\dl]. These results were agreement with other study as [17]. Androgen appears to produce a change in the hormonal

profile of animals, primarily due to elevated androgen levels. This raised testosterone levels produced insulin resistance, therefore causing a lower glucose tolerance.

Impact of management of Glyrrhiza Glabra L. root extract on blood sugar, was shown in Table 3, which indicate that the root wad significantly decreases the level of glucose and this result was conformable with other study [35]. The action of providing licorice root hydroalcoholic extract on blood serum glucose, TG, and TC concentration had been studied in 50 Sprague rats on PCOS, which they founded that the letrozole group had higher blood serum glucose than normal control [$p<0.05$]. Thus, licorice root extract decreases unfavorable consequences of diabetes caused by PCOS [36]. Furthermore, green tea extract may have therapeutic advantages for PCOS due to its antioxidant and anti-inflammatory properties. The comprehensive study investigates the possible impacts of Camellia sinensis on metabolic factors, hormone and function of ovarian in PCOS [37]. The current study›s results indicate that marjoram extract had a good effect in the hormonal profile of PCOS women, it progresses sensitivity of insulin also, lowers adrenal androgen levels.

At the same time, metformin lowered the level of glucose considerably if compared to the PCOS group. Metformin therapy may reduce glucose resistance through preserving glucose homeostasis and enhancing insulin-mediated glucose absorption. [38]. Androgen significantly lowers glucose levels in medicated groups when compared to PCOS group due to its antihyperglycemic effect. It may play a role in potentiating insulin secretion by the β-cells, supports regulated absorption of glucose by cells. It further postulated the androgens may be a potential glycemic control medication by increasing insulin-dependent receptor kinase function, as a result, increasing glucose transporter-4 translocation and uptake. The results of our research about the total protein were identical with other studies such as [17]. TP increased considerably in PCOS group. The excessive total protein concentration may suggest PCOS group, as well as viral and chronic inflammation due to abnormal androgen production. Yet, metformin dramatically reduced the amount of TP in comparison to healthy control group. Metformin medication leads to enhanced sensitivity of proteins and protein hormones, which decreases the quantity of protein in blood. The flavonoid components found in Glyrrhiza glabra L., Camellia sinensis, and Origanum majorana extracts dramatically reduced total protein levels by suppressing eicosanoids and cytokines [17].

One of the variables that was measured in our study is CRP, found that there wasn›t a significant distinction across infected group and rest of groups. CRP is an acute form of protein that serves as an early sign of tissue inflammation. It is a renowned infection marker, but it can also be used to monitor low-grade chronic inflammation and anticipate vascular events. Other research has found that PCOS have higher CRP, indicating that the illness is chronically inflammatory [40,41].

The peroxidation of lipid is the free-radical-mediated propagation of oxidative damage in polyunsaturated fatty acids, including a variety of free radicals. Antioxidants inhibit lipid peroxidation via enzyme activity, or activity of the free radical scavenging. An rise in lipid per hydroxide levels might indicate attributed to terminate the antioxidant action that triggered the progression of the disease [34].

Nature has consistently been a good supplier of numerous therapeutic compounds, providing us with several medicinal plants that create beneficial molecules. As a consequence, the market for medicinal plants and wellness products keeps expanding. From these plant Glyrrhiza glabra L., Camellia sinensis, and Origanum majorana. The phytochemical ingredients and pharmacological influence of those compounds on these plants determined their effectiveness. Multiple compounds in these plants, can provide us with pharmacological benefits [42].

Metformin substantially lowered MDA levels, raised GSH concentrations, and boosted GSH-Px activity in comparison to PCOS group. These findings may be referred to metformin›s complexity, as it has several sites of action and molecular processes. Metformin operates to reduce glucose synthesis, enhancing insulin sensitivity and on the stomach to enhance glucose utilisation, and microbiome composition [43].

The results of liver enzymes activities were identical with other study [44], which they indicated a greater activity of ALT in PCOS patients, ascribed mostly to growing prevalence of obesity, hyperandrogenism, insulin resistance, and dyslipidemia associated with PCOS. Nevertheless, association in ALT and PCOS with obese situation had been documented more often

compared to overweight PCOS. Liver function test isn't indicated consistently until patient is obese or overweight. it could lead these patients to lose out on best chances for intervention, like modifying their lifestyle and check in on a regular basis to avoid or postpone the onset of the condition. Also, [45] note that PCOS group revealed obvious rises in activities of serum alanine transaminase and aspartate aminotransferase, and we found that steatosis of liver in PCOS rats was related with hyperandrogenemia. Androgens can aggravate the hepatic TG accumulation by reducing microsomal triglyceride transfer protein expression in PCOS.

There is emerging evidence that hyperandrogenism is linked to PCOS. Androgen excess may contribute to PCOS in patients by directly affecting the liver, indirectly modulating insulin sensitivity and secretion, increasing visceral obesity, or a combination of these actions. Insulin resistance, in consequence, affects the synthesis, clearance, and bioavailability of ovarian androgens, which contributes significantly to ovarian androgen excess [46].

Giving the Glyrrhiza Glabra L., Camellia Sinensis, and Origanum majorana extracts in addition of metformin as drug enhances the effectiveness of the enzyme's activity, as we found that they all decreased in comparison with PCOS group, evidence of an improvement in the disease condition.

Our results of sex hormones were symmetric with other study [47]. Establish plants that are currently being studied for its effects in POCS when studies on human subjects and animal models. The majority of research studied the concentration of sex hormones, insulin resistance, hyperandrogenism, ovulation, and PCOS symptoms before and after treatment.

Creating polycystic ovarian syndrome and then treating with different extracts, reduced testosterone concentration while increasing progesterone were showed in blood serum. These studies show the examined herbal extracts were beneficial to treating PCOS, improved levels of sex hormones, and PCOS symptoms [31]. In women who suffer from PCOS, the synthesis and metabolism of androgens, estrogens are disturbed, and androgen levels increase. On the appearance of insulin resistance, there is highly increase in insulin. As a consequence of insulin resistance, LH/FSH levels rise, and hormone alterations at theca cell surface and granulosa boost androgen synthesis while decreasing estradiol synthesis. Lastly, follicular maturation is halted, resulting in compromised ovulation. Because medicinal plants contain active chemicals and have no significant bad effects, they have gotten an abundance of publicity recently. As an example, Glyrrhiza glabra L., Camellia sinensis, and Origanum majorana are some of these plants that have high levels of phytoestrogens. This plant›s anti-androgenic properties cause a reduction in androgen levels in PCOS patients [48].

Raised testosterone levels in PCOS are most likely due to an accumulation of androgens caused by an aromatase inhibitor, which inhibited the conversion of androgen substrates into oestrogen. The drop in testosterone levels in metformin group reflects decreased androgen production in ovary [17]. Metformin, an insulin lowering medication, proved popular in PCOS after it was discovered that insulin resistance played a significant role in the disorder›s etiology.

We note that the weight of the hearts is non-significantly differences between the study groups, the reason that may be the period of time taken by the study after the development of the pathological condition, which was 28 days, is few, and that weight gain due to polycystic ovaries is likely to take longer to appear significantly. Table 7, indicate that there were non-significantly changes between the PCOS and control groups in final body weight. Also, between the experiment groups. These results were identical with [39] which they note that there were no changes in laurel plant extract group when compared to control normal group.

CK activity was substantially higher in the PCOS group than in the control group. This outcome was similarly to previous research [49]. Creatine kinase is an enzyme presents primarily in the brain, skeletal muscles, and heart. CK levels are raised after cardiac attacks, when the muscle of heart is injured. Trauma and other diseases that injure the skeletal muscle are also linked to increased CK activity. In rare situations, the test can be used to detect muscle diseases such polymyositis [muscle inflammation] or to determine the extent of muscle damage. Serum creatine kinase activity is an important aspect in evaluating muscular weakness or myalgia, as well as diagnosing myopathies or rhabdomyolysis. However, increased CK can be an accidental finding without muscle-related symptoms or with relatively minor nonspecific muscular symptoms

that do not significantly interfere with activities of daily living [50].

The conclusion of LDH activity in cardiac tissues was consistent with earlier studies [51]. LDH is an essential enzyme that aids in cellular respiration, the process by which the body converts glucose from meals into energy for its cells. It was found in practically all tissues in the body. As new cells grow in tissues, the body eliminates older, or «dead» ones. This is a typical process in which tissues release LDH into the circulation or other bodily fluids. On the other hand, LDH has been demonstrated to be a sign of tissue necrosis in other emergent situations such as myocardial and intestinal infarction, therefore the use of LDH as a diagnostic for ovarian torsion is feasible [51].

The IL-1β, was measured in heart tissue of female rats with POCS and we discovered an extremely significant rise in the impacted group compared with healthy control group. Resveratrol, an anti-inflammatory and anti-hyperglycemic drug, could be lower the risk of diabetes in PCOS patients by reducing the production of pro-inflammatory cytokines TNF-α and IL-1β. Our study›s results were consistent with prior research [52-53].

PCOS is a complicated multifactorial condition that interested both hereditary and climate components. IL-1β, a cytokine that regulates immunological and inflammatory responses, has been linked to PCOS as a hereditary factor. As a result, deregulation of such immunological activity may contribute to pathogenesis of PCOS. A single nucleotide polymorphism [SNP] in the Interleukin IL-1β gene promoter may regulate IL-1β levels. Furthermore, this SNP may affect the expression of this cytokine in PCOS patients. Researchers have found a link with IL-1β concentration and PCOS obesity patients [52].

Giving aqueous extracts to animals infected with polycystic ovaries improved the effectiveness of the CK and LDH activities and IL-1β, as the results in Table 8 show that marjoram extract gave less effectiveness of the enzymes compared to affected group, and the result was close to the control group.

Metformin significantly reduced the activities of CK, LDH and IL-1β when rapprochement to PCOS group. These results could be attributed to metformin which is a complicated medication with several sites of action and molecular processes [43].

Table 8 suggested an extremely substantial rise in Tumor Necrosis Factor-alpha of PCOS group in comprise with normal group. The results of our current study are similar to those found [54]. TNF-α has been connected to PCOS-related symptoms like hyperandrogenism, resistance of insulin, and obesity. TNF-α overexpression in muscle and adipose tissues has been linked to the development of reduces insulin receptor tyrosine kinase activity, causes hyperandrogenism, and influences follicular development, making it a potential contributor to PCOS. None of the Glyrrhiza glabra L., Camellia sinensis, and Origanum majorana extracts was given as well as a metformin any significant change compared to the group with PCOS.

Conclusion

In conclusion, our study has demonstrated that marjoram [M], green tea [GT], and liquorice root [L.] can decrease the negative effects of PCOS in particular on cardiovascular features, and treating PCOS with these herbs is recommended. Results indicated a considerable decline in IL-1β in the heart tissue of the groups treated with the extracts, where the highest decrease was in the group treated with marjoram extract compared to the negative control group, followed by green tea and finally licorice roots, which are good drop ratios compared to metformin as a standard treatment. Groups 3 to 6 showed a notable improvement in cardiac tissue activity for both CK and LDH, with the fifth group treated with marjoram showing the highest activity levels for both CK and LDH, respectively, compared to the negative control group.

Acknowledgment

The authors would like to thank the stuff of college of Veterinary Medicine, Mosul University for their help and support in performing the research.

Funding statement

None.

Inconsistency of interests

For conducting this work, the authors did not receive any compensation or benefits from the industry, funding organizations, or any other source.

References

1. Mohammed, Z. A. A study of pathological abnormalities of genitalia in ewes in Duhok, Iraq. *Iraqi Journal of Veterinary Science*, **35** (3), 421-427(2021).DOI: 10.33899/ijvs.2020.126939.1421.

2. Yasmin, A., Roychoudhury, S., Paul Choudhury, A., Ahmed, A.F., Dutta, S. and Mottola, F. Polycystic ovary syndrome: An updated overview foregrounding impacts of ethnicities and geographic variations. *Life*, **12** (12),1974(2022). DOI: 10.3390/life12121974.

3. De Leo, V., Musacchio, M.C., Cappelli, V., Massaro, M.G., Morgante, G. and Petraglia, F. Genetic, hormonal and metabolic aspects of PCOS: an update. *Reprod. Biol. Endocrinol*., **16**(1),38(2016). DOI: 10.1186/s12958-016-0173-x. PMID: 27423183; PMCID: PMC4947298.

4. Mohammadi, M. Oxidative stress and polycystic ovary syndrome: A brief review. *International Journal of Preventive Medicine*, **10**,86(2019). DOI: 10.4103/ijpvm.IJPVM_576_17.

5. Sengupta, P., Dutta, S. and Hassan M.F. Polycystic ovary syndrome [PCOS] and oxidative stress. *Journal of Integrated Science and Technology*, **12** (3), 752. (2024). DOI:10.62110/sciencein.jist.2024.v12.752.

6. Darabi, P., Khazali, H. and Natanzi, M. M. Therapeutic potentials of the natural plant flavonoid apigenin in polycystic ovary syndrome in rat model: via modulation of pro-inflammatory cytokines and antioxidant activity. *Gynecological Endocrinology*, **36**(7),582-587 (2019). DOI:10.1080/09513590.2019.1706064.

7. Yifu, P. Evidence for causal effects of polycystic ovary syndrome on oxidative stress: a two-sample mendelian randomisation study. *Yifu BMC Medical Genomics*, **16**,141(2023). https://doi.org/10.1186/s12920-023-01581-0.

8. Agarwal, A. and Sengupta, P. Oxidative stress and its association with male infertility. Male infertility: contemporary clinical approaches. *andrology, ART and Antioxidants*, **12** , 57-68(2020). DOI:10.1007/978-3-030-32300-4_6.

9. Malini, N. and George. K.R. Evaluation of different ranges of LH: FSH ratios in polycystic ovarian syndrome [PCOS]–Clinical based case control study. *Gen. Comp. Endocrinol.*, **260**, 51-57(2018). DOI: 10.1016/j.ygcen.2017.12.007.

10. Moghetti, P. and Tosi, F. Insulin resistance and PCOS: chicken or egg? *J. Endocrinol. Invest.*, **44**, 233-244(2021). DOI: 10.1007/s40618-020-01351-0.

11. Kalhori, Z., Azadbakht, M. Soleimani Mehranjani, M. and Shariatzadeh, M.A. Improvement of the folliculogenesis by transplantation of bone marrow mesenchymal stromal cells in mice with induced polycystic ovary syndrome. *Cytotherapy*, **20**(12), 1445–1458(2018). DOI: 10.1016/j.jcyt.2018.09.005.

12. Beazley, K.F. and Nurminskaya, M.J.R. Effects of dietary quercetin on female fertility in mice: implication of transglutaminase 2. *Reprod. Fertil. Dev*., **28**(7), 974-981(2016). DOI: 10.1071/RD14155

13. Hameed, O.M., Sukayna H. R. and Al-Helaly, L. A. Changes in the Level of Zinc and Copper and Some Biochemical Parameters in Patients with Chronic Kidney Failure. *Biomedical. and Biotechnology Research Journal,* **7**(1), 118-122(2023). | DOI: 10.4103/bbrj.bbrj_22_23

14. Hamdoon, A.A., Al-khashab E.M. and Al-Hashemi, H.M. Effect of saponin extract of *Glycyrrihiza glabra* in activity of hepatic enzymes and some biochemical parameters in serum of adults ovariectomized female rats. *Iraqi Journal of Veterinary Sciences*, **34**(2), 411-415(2020). DOI: 10.33899/ijvs.2019.126127.1239,

15. Mombaini, E. The Impact of Green Tea Supplementation on Anthropometric Indices and Inflammatory Cytokines in Women with Polycystic Ovary Syndrome. *Phytother. Res*., **31**(5), 747-754(2017). DOI: 10.1002/ptr.5795.

16. 16. Bina, F. and Rahimi, R. Sweet Marjoram: A Review of Ethnopharmacology, Phytochemistry, and Biological Activities. *Journal of Evidence-Based Complementary & Alternative Medicine*, **22** (1),175-185. (2017). https://doi.org/10.1177/2156587216650793 .

17. Jahan, S., Munir, F., Razak, S., Mehboob, A., Ul-Ain, Q., Ullah, H., Afsar, T., Shaheen, Gh. and Almajwal, A. Ameliorative effects of rutin against metabolic, biochemical and hormonal disturbances in polycystic ovary syndrome in rats. *Journal of Ovarian Research*, **9**(86),(2016). https://doi.org/10.1186/s13048-016-0295-y .

18. Al-Abachi, S.Z. and Al-Gorany, S.M. (2019). Effects of Crude Aqueous Extract of the Palmito on some Biochemical Parameters in Serum of Healthy Rats. The Eurasia Proceeding of Science, Technology, Engineering & Mathematise [EPSTEM], **7**, 321-328. *International Conference on Technology, Engineering and Science [IConTES]*, (2019), Antalya/Turkey.

19. Rashed, S.H., Al-Ameen, S.A. and Matti, M.G. Some biochemical effects of Aloe vera leaves on tissues in normal mice. *Iraqi Journal of Veterinary Sciences*, **24**(2), 93-97(2010).doi: 10.33899/ijvs.2010.5605.

20. Jasim, W.K., Mosa, A.U., Ouda, M.H. and Mousa, R.F. Curative role of silymarin against reproductive toxicity of the female system during the critical periods of life. *Iraqi Journal of Veterinary Sciences*, **37**, 183-189(2023). DOI: 10.33899/ijvs.2023.138618.2817.

21. Marcondes, K.F., Bianchi, F.J. and Tanno, A.P. Determination of the estrous cycle phases of rats: some helpful considerations, *Brazilian Journal of Biology*, **62**(4A), 609–614(2002). DOI: 10.1590/s1519-69842002000400008.

22. Bradic, J., Zivkovic, V., Srejovic, I., Jakovljevic, V., Petkovic, A., Turnic, T.N., Jeremic, J., Jeremic, N., Mitrovic, S., Sobot, T., Ponorac, N., Ravic, M. and Tomovic, M. Protective effects of Galium verum L. extract against cardiac ischemia/reperfusion injury in spontaneously hypertensive rats, *Oxidative Medicine and Cellular Longevity*, **2019**, Article ID 4235405, 11 pages, (2019). DOI: 10.1155/2019/4235405.

23. Al-Abachi, S. Z., Yaseen, S. M. and Shihab, Gh. A. Biochemical study of consumption Zahdi dates [*Phoenix daetifera*] in type 2 diabetic patients. *Rafidain Journal of Science*, **31**(1), 11-22(2022). DOI: 10.33899/rjs.2022.172924.

24. Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. Estimation of the concentration of lowdensity lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, **18**(6), 499-502(1972). PMID: 4337382.

25. Wilson, P.W., Abbott, R.D., Garrison, R.J. and Castelli, W.P. Estimation of Very –Low Density Lipoprotein Cholesterol from data on triglycerides concentration in plasma. *Clin. Chem. J*., **27**(12), (1981). 2008-10. PMID: 6946880.

26. Mohandas, J., Marshall, J.J., Duggin, G.G., Horvath, J.S. and Tiller, D.J. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: possible implications in analgesic nephropathy. *Biochem. Pharmacol.*, **33**(11),1801–1807(1984). https://doi.org/10.1016/0006-2952[84]90353-8.

27. Islam, H., Masud, J., Islam, Y.N. and Haque, F.K.M. An update on polycystic ovary syndrome: A review of the current state of knowledge in diagnosis, genetic, etiology, and emerging treatment options. *Women's Health*, **18**, 1–23(2022). DOI: 10.1177/17455057221117966.

28. Prajapati, P. A lipid profile study in polycystic ovary syndrome women. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, **12**(1),234-239(2023). DOI:10.18203/2320-1770.ijrcog20223500.

29. Kiranmayee. D., Kavya, K., Himabindu, Y., Sriharibabu, M., Madhuri, G.L.J. and Venu, S. Correlations between anthropometry and lipid profile in women with PCOS. *J. Hum. Reprod. Sci.*, **10**(3),167-72(2017). DOI: 10.4103/jhrs.JHRS_108_16.

30. Teede, H.J., Misso, M.L. and Costello, M.F. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Hum. Reprod.*, **33**(9), 1602–1618(2018). DOI: 10.1093/humrep/dey256.

31. Abasian, Z., Rostamzadeh, A., Mohammadi, M., Hosseini, M. and Rafieian-kopaei, M. A review on role of medicinal plants in polycystic ovarian syndrome: Pathophysiology, neuroendocrine signaling, therapeutic status and future prospects. *Middle East Fertility Society Journal*, **23** (4), 255-262(2018). https://doi.org/10.1016/j.mefs.2018.04.005.

32. Andhalkar, S., Chaware, V. and Redasani, V. A Review on Medicinal Plants of Natural Origin for Treatment of Polycystic Ovarian Syndrome [PCOS]. *Asian J. Pharm. Res. Dev.,* **9**, 76-81(2021). DOI: 10.22270/ajprd.v9i3.949.

33. Notaro, A.L.G. and Neto, F.T.L. The use of metformin in women with polycystic ovary syndrome: an updated review. *Journal of Assisted Reproduction and Genetics*, **39**,573–579(2022). doi: 10.1007/s10815-022-02429-9.

34. Ohkawa, H., Ohishi, N. and Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Analytical Biochemistry*, **95**(2),325–358(1979). DOI: 10.1016/0003-2697[79]90738-3.

35. Manouchehri, A., Abbaszadeh, S., Ahmadi, M., Nejad, F.Kh., Bahmani, M. and Dastyar, N. Polycystic ovaries and herbal remedies: A systematic review. *JBRA Assisted Reproduction*, **27**(1), 85-91(2023). DOI: 10.5935/1518-0557.20220024.

36. Barazesh, F., Mirzaei, A., Abbasian, Z. and Ghavami, Z.M. The Effect of Hydroalcoholic Extract of Glycyrrhizaglabra L. [liquorice] Root on Serum Level of Glucose, Triglyceride, and Cholesterol in Polycystic Ovary Syndrome Induced by Letrozole in Rats. *Armaghan Danesh.*, **21**,148-59(2016).

37. Maleki, V., Taheri, E., Varshosaz, P., Tabrizi, F.P.F., Moludi, J., Jafari-Vayghan, H., Shadnoush, M., Jabbari, S.H.Y., Seifoleslami, M. and Alizadeh, M. A comprehensive insight into effects of green tea extract in polycystic ovary syndrome: a systematic review. *Reprod. Biol. Endocrinol*., **23**,19(1),147. (2021). DOI: 10.1186/s12958-021-00831-z.

38. Pai, S.A. and Majumdar, A.S. Protective effects of melatonin against metabolic and reproductive disturbances in polycystic ovary syndrome in rats. *J. Pharm. Pharmacol.*, **66**(12), 10-21(2014). DOI: 10.1111/jphp.12297.

39. Abdullah, A. I. and Al-Abachi, S. Z. Study of the laurel plant [Laurus Nobilis] on some biochemical markers in diabetic mellitus rats. *Egyptian Journal of Chemistry*, **64**(11), 6529-6540(2021). DOI:10.21608/ejchem.2021.79203.3889.

40. Gözüküçük, M., Gürsoy, A.Y., Destegül, E., Taşkın, S. and Şatıroğlu, H. Homocysteine and C-reactive Protein Levels in Women with Polycystic Ovary Syndrome. *Gynecol Minim Invasive Ther.*, **10** (4), 210–214(2021). DOI:10.4103/GMIT.GMIT_30_20.

41. Hashemian, Z. and Afsharian, P. Role of Oxidative Stress in Polycystic Ovary Syndrome. *Journal of Shahid Sadoughi University of Medical Sciences*, **28**(5),2634-2647(2020). DOI:10.18502/ssu.v28i5.3972.

42. Hasan, M.K., Ara, I., Mondal, M.Sh.A. and Kabir, Y. Phytochemistry, pharmacological activity, and potential health benefits of Glycyrrhiza glabra. *Heliyon*., **7**(6), e07240(2021). DOI:10.1016/j.heliyon.2021.e07240.

43. Rena, G., Hardie, D.G. and Ewan, R. The mechanisms of action of metformin. *Diabetologia,* **60**,1577–1585(2017). DOI: 10.1007/s00125-017-4342-z.

44. Liu, C., Liu, K., Zhao, X., Zhu, J., Liu, Y., Hao, L., Gao, Y. and Liu, P. The Associations Between Alanine Aminotransferase and Other Biochemical Parameters in Lean PCOS. *Reprod. Sci.*, **30**(2), 633-641(2023). DOI: 10.1007/s43032-022-01030-w. Epub 2022 Jul 21. PMID: 35864417; PMCID: PMC9988735.

45. Wang, D., Zhao, H., Xing, Ch., Lv, B., Wang X. and He, B. Androgens exacerbate hepatic triglyceride accumulation in rats with polycystic ovary syndrome by downregulating MTTP expression. *Endocrine*, 2023 Nov **11**. [2023]. DOI: 10.1007/s12020-023-03590-6. Online ahead of print.

46. Vassilatou, E. Nonalcoholic fatty liver disease and polycystic ovary syndrome. *World J. Gastroenterol.*, **20**(26), 8351–8363(2014). DOI: 10.3748/wjg.v20.i26.8351

47. Ranjbary, A.G., Mehrzad, J., Garoussi, M.T. and Zohdi, J. Long Term Oral Administration of Oregano Essence Effectively Relieves Polycystic Ovarian Rats through Endocrine and Inflammatory Balance. *Hindawi Evidence-Based Complementary and Alternative Medicine*, **2022**, Article ID 5303583, 12 pages. (2022). https://doi.org/10.1155/2022/5303583.

48. Rajan, R.K. and Balaji, B. Soy isoflavones exert beneficial effects on letrozole-induced rat polycystic ovary syndrome [PCOS] model through anti-androgenic mechanism. *Pharm. Biol*., **55**, 242-251(2017). DOI: 10.1080/13880209.2016.1258425

49. Al-Hail, N., Butler, A.E., Dargham, S.R., Abou Seif, A. and Atkin, S.L. Creatine Kinase is a Marker of Metabolic Syndrome in Qatari Women with and without Polycystic Ovarian Syndrome. *Frontiers in Endocrinology*, **10**, 659-666(2019). https://doi.org/10.3389/fendo.2019.00659.

50. Moghadam-Kia, S., Oddis, Ch. V. and Aggarwal, R. Approach to asymptomatic creatine kinase elevation. *Cleve . J. Med*., **83**(1), 37–42(2016). DOI:10.3949/ccjm.83a.14120.

51. Mayhew, A., Patel, N., Hanfling, S., Dowlut-McElroy, T. and Gomez-Lobo, V. Lactate Dehydrogenase as a Marker of Ovarian Torsion. *Journal Pediatric and Adolescent Gynecology*, **34** (2), P243(2021). DOI:https://doi.org/10.1016/j.jpag.2021.02.013.

52. Saleh-Gargari, S., Ghafouri-Fard, S., Zandieh, Z., Ansaripour, S., Pouresmaeili, F. and Vatannejad, A. The rs16944 SNP in IL-1B and risk of polycystic ovarian syndrome. *Gene Reports.* **17**,100547(2019). https://doi.org/10.1016/j.genrep.2019.100547.

53. Ghowsi, M., Khazali, H. and Sisakhtnezhad, S. Evaluation of TNF-α and IL-6 mRNAs expressions in visceral and subcutaneous adipose tissues of polycystic ovarian rats and effects of resveratrol, *Iranian Journal of Basic Medical Sciences*, **21**(2),165–174(2018). DOI:10.22038/IJBMS.2017.24801.6167

54. Thathapudi, S., Kodati, V., Erukkambattu, J., Katragadda, A., Addepally, U. and Hasan, Q. Tumor Necrosis Factor-Alpha and Polycystic Ovarian Syndrome: A Clinical, Biochemical, and Molecular Genetic Study. *Genetic Testing and Molecular Biomarkers,* **18**(9), 605–609(2014). DOI: 10.1089/gtmb.2014.0151.

دور الإجهاد التأكسدي على الخصائص القلبية الوعائية لمتلازمة تكيس المبايض المستحدث بالأندروجين عند اناث الجرذان

سكينة حسين رشيد[١] ، صبا زكي العباجي[١] و رغد عبد الموجود محمد حمو[١]
قسم الكيمياء- كلية العلوم- جامعة الموصل- الموصل- العراق.

ان متلازمة المبيض متعدد الكيسات عولج بمستخلصات كل من جذور عرق السوس [١٥٠ ملغم/كغم من وزن الجسم] و الشاي الاخضر [٢٥٠ ملغم/كغم من وزن الجسم] و المردقوش [١٠٠ ملغم/كغم من وزن الجسم] لمدة ٢٨ يوما. كذلك استخدم المتفورمين [٢٠ ملغم/كغم من وزن الجسم] كدواء قياسي. الهدف من الدراسة هو تقييم المتغيرات الكيموحيوية في مصل الدم وأنسجة القلب في أناث الجرذان لمتلازمة المبيض المتعدد الكيسات المستحدث بالأندروجين ودوره في تطور الاضرار القلبية الوعائية الناتجة عن المتلازمة لاكتشاف أدوية جديدة بأقل قدر من السمية ، وهذا يمثل تحديا علميا واسعا. استخدمت ٣٦ من اناث الجرذان البيضاء بواقع ستة مجاميع كل واحدة تضم ٦ جرذان. ان المجاميع المعاملة بمستخلصات جذور عرق السوس والشاي الاخضر والمردقوش أدت الى تحسين مستويات المتغيرات الكيموحيوية ومنها قد قاربت قيم المستويات الطبيعية. كذلك تبين ان المجموعة التي عوملت بالمستخلص المائي للمردقوش أعطت اكثر فعالية من المجاميع التي عوملت بكل من مستخلصي جذور عرق السوس والشاي الاخضر وقد عملت على تحسين الخصائص القلبية الوعائية.

ابرز الاستنتاجات التي توصلت اليها الدراسة الحالية هي انخفاض تراكيزHDL-C بمقدار 45.4% بعد مرور ٢٨ يوما فقط من اصابة اناث الجرذان بتكيس المبيضين. كما ان المستخلصات الثلاثة سببت حصول ارتفاعا في الاسترادول والبروجستيرون وبنسب مرتفعة [110.3%و77.6%] على التوالي. اما فيما يخص نسيج القلب فقد عادت فعالية انزيم الكرياتين كاينيز واللاكتيت ديهيدروجنيز والانترليوكين -١بيتا الى معدلاتها الطبيعية للمجاميع المعالجة بالمستخلصات المائية الثلاثة حيث انها لم تعط اختلافات معنوية عند مقارنتها مع قيم السيطرة الطبيعية. ان المستخلص الاكثر تأثيرا عل مستوى الهرمونات في مصل الدم هومستخلص الشاي الاخضر يليه مستخلص المردقوش ومن ثم مستخلص جذور عرق السوس.

الكلمات المفتاحية: الأندروجين، الشاي الأخضر، جذر عرق السوس، المردقوش، متلازمة المبيض المتعدد الكيسات.