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"Impact of L-Arginine and Licorice Extract Supplementation on Growth and Epiphyseal Cartilage Health in PCOS-Induced Rats"

Yasmeen El-Sayed [a], Heba A. El-Ghaweet[a], Abdelaziz Elrefaeey[b], Amoura M. Abou-El-Naga[a]

[a] *Faculty of Science, Mansoura University, Zoology Department, Mansoura, Egypt.*

[b] *Faculty of Medicine, Mansoura University, Obstetrics and Gynecology Department, Mansoura, Egypt.*

**** Correspondence to:** yasmeensayed522@mans.edu.eg Tel: 01018502820)*

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Abstract: Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder characterized by hyperandrogenism and ovarian dysfunction, often leading to metabolic and reproductive complications. Due to their antioxidant and anti-inflammatory properties, L-arginine and licorice extract have been explored for their potential in infertility management. This study examines the effects of L-arginine and licorice extract on body weight and epiphyseal cartilage morphology in a rat model of PCOS. Forty-eight female rats were divided into six groups (n = 8): Group I (Control) received no treatment; Group II (Licorice) was administered 150 mg/kg licorice extract for 21 days; Group III (L-Arginine) received 3.5 mg/kg L-arginine for 21 days; Group IV (PCOS Model) was induced using 1 mg/kg letrozole for 21 days; Group V (Licorice + PCOS) received licorice extract following PCOS induction; and Group VI (L-Arginine + PCOS) was treated with L-arginine after PCOS induction. The study assessed body weight, absolute weights of the genital system, uterus, and ovaries, along with the histological structure of epiphyseal cartilage. Both L-arginine and licorice extract significantly improved epiphyseal cartilage integrity and regulated body weight, suggesting their potential therapeutic role in PCOS management.

keywords: Polycystic Ovary Syndrome, L-arginine, Licorice Extract, epiphyseal cartilage, and Body Weight.

1.Introduction

Polycystic ovarian syndrome (PCOS) is a prevalent endocrine disorder affecting 5-10% of women of reproductive age. It is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries, often leading to complications such as insulin resistance, obesity, and dyslipidemia [1]. These metabolic disturbances significantly increase the risk of type 2 diabetes and cardiovascular diseases. Traditional treatment approaches, including hormonal contraceptives and insulin sensitizers, primarily manage symptoms but do not address the underlying pathophysiology, and they often come with adverse effects, highlighting the need for alternative therapeutic options [2].

Licorice (Glycyrrhiza glabra) has a long history of use in traditional medicine due to its anti-inflammatory, antioxidant, and endocrine-modulating properties [3]. The active constituents of licorice, such as glycyrrhizin and flavonoids, have demonstrated potential in modulating hormonal and metabolic disturbances associated with PCOS[4]. Recent studies suggest that licorice extract may exert beneficial effects on insulin sensitivity, androgen levels, and inflammatory pathways in PCOS models [5].

L-arginine, a semi-essential amino acid, is a precursor for nitric oxide, which is crucial for vascular function and insulin sensitivity [6]. Studies have suggested that L-arginine supplementation can improve insulin resistance, reduce oxidative stress, and positively influence metabolic profiles [7].

This study aims to investigate the therapeutic efficacy of aqueous licorice extract and L-arginine in alleviating PCOS-related metabolic and skeletal abnormalities. By utilizing an experimental animal model, this research seeks to elucidate the underlying

mechanisms through which these compounds influence body weight regulation and epiphyseal cartilage integrity, thereby providing a scientific basis for their potential application as alternative therapeutic agents in PCOS management.

2. Materials and methods

2.1. Chemicals

-Letrozole and L-arginine were purchased from Sigma-Aldrich, Saint Louis, MO, USA.

-Licorice was sourced locally from markets in Iraq.

2.1. Preparation of Licorice Extract:

The dry powder of Licorice was purchased from Iraq. The collected samples were identified and authenticated by the expert Botanical Laboratory, Department of Botany, Faculty of Science, Mansoura University, Egypt. In glass flask, the powder of the dried licorice was extracted, 20 gm of the plant powder was settled in a conical flask (250 ml), and then 100 ml of distilled water were added. The conical flasks were kept on a horizontal water bath shaker at 70 °C for 30 minutes at 200 rpm. The extracts mixtures were settled until reach to room temperature then filtered using Whatman filter paper No. 1 (Whatman Int. Ltd., Kent, UK) using a Buchner funnel. The concentration of the obtained extract was determined and the extracted solution was stored at 0-5 oC [8]. The extracts were kept in sterile bottles, under refrigerated conditions, until further use.

2.2. Experimental animals

48 healthy adult female Sprague Dawley (SD) rats, aged 6-7 weeks with an average weight of 150 g, were used in this study. The rats were sourced from the Egyptian Vaccine Company (VACSERA, Giza, Egypt) and housed in stainless steel cages at the animal house of the Zoology Department, Faculty of Science, Mansoura University, Egypt.

The rats were maintained under controlled conditions, with a constant temperature of 22-25°C, a 12-hour light/dark cycle, 50-60% humidity, and access to a standard diet and water ad libitum. The study was conducted following the approval of the Mansoura University Animal Care and Use Committee (MU-ACUC) with **code NO. : MU-ACUC (SC.MS.23.05.24)**.

2.2.1. Experimental design:

Experimental animals were divided randomly and equally into six groups (n = 8rats/group), as thefollowing:

Group I (**Control**): Normal healthy animals were received saline solution orally for 21 days.

Group 2 (Licorice group): Animals were received orally 150 mg/kg Licorice extract daily for 21 days via gastric tube.

Group 3 (L-arginine group): Animals were received orally 3.5mg of L-arginine extract per rat daily for 21 days.

Group 4 (Induced Polycystic Ovary Syndrome (PCOS) - group): PCOS was induced by administering 1 mg/kg letrozole orally once daily for 21 days [9].

Group 5 (PCOS + Licorice group): PCOS-induced rats received 150 mg/kg licorice extract orally once daily for 21 days following PCOS induction [10].

Group 6 (PCOS + L-arginine group): PCOS-induced rats received 3.5 mg/rat L-arginine orally once daily for 21 days following PCOS induction [11].

All treatments were administered via oral gavage to ensure precise dosing.

2.2.2. Animal investigations

2.2.2.1. Body weight: During the study, the body was weighted every day for all groups, and recorded.

2.2.2.2. Tissue sampling

The rats were dissected, and the ovaries, uterus and epiphyseal cartilage were carefully removed. The organs were weighed, and the organ-to-body weight ratio was calculated. The epiphyseal cartilage was preserved in buffered formalin for histological investigation.

Statistical analysis

All the grouped data were statistically analyzed using GraphPad Prism 8.0.1 software, using one-way ANOVA. All the results were recorded as the mean ± SD, where statistically significant data were considered at $p < 0.05$.

3. Results

3.1. Body weight of the control and other experimental groups

The mean body weight of all experimental groups was measured weekly throughout the study period and is presented in **Figure 1**. Rats in the PCOS group exhibited a significant increase in body weight ($P \leq 0.001$) compared to the control group. However, treatment with L-arginine or licorice extract resulted in a significant reduction in body weight in polycystic female rats compared to the untreated PCOS group.

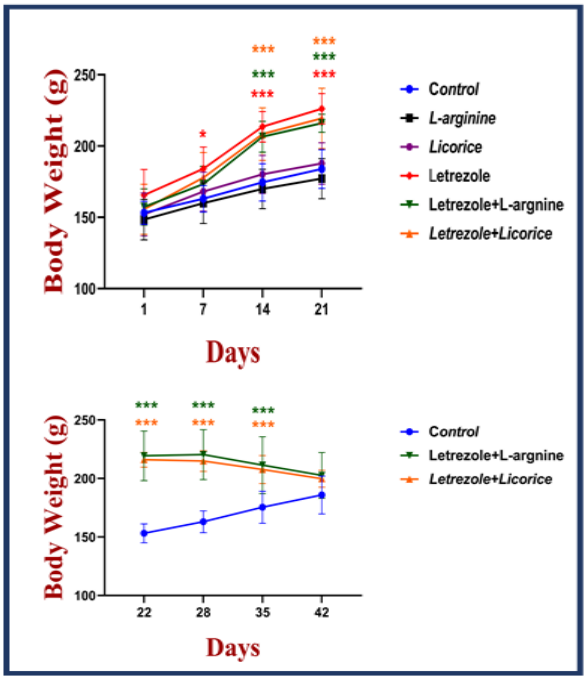

Fig.1. The bodyweight (g) changes in different experiment groups.

- Data illustrated as Mean ± SEM, n = 6
- *P < 0.05 and ***P < 0.001 compared to control group
- Cont: Control; Letrezole: PCOS group.

3.2. Absolute female genital system weights of the control and other experimental groups

Figure (2) illustrates the changes in female genital system weight across the control and experimental groups. The results demonstrated a significant reduction in genital system weight in the PCOS group ($P \leq 0.001$) compared to the control group. However, treatment with L-arginine or licorice extract led to a significant increase in genital system weight in polycystic female rats compared to the untreated PCOS group

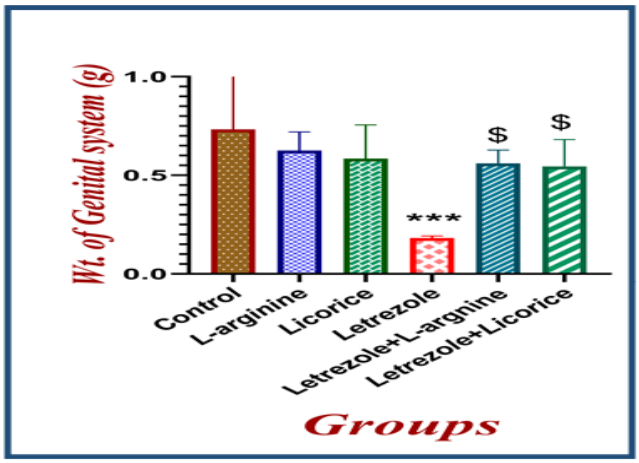

Fig.2: Female genital system weight changes (g) in the control and other experimental groups.

Data illustrated as Mean ± SEM, n = 6

- $^{***}P < 0.001$ compared to control group
- $^{\$}P < 0.05$ compared to PCOS group
- Cont: Control; Letrezole: PCOS group.

3.3. Absolute Uterus weights of the control and other experimental groups

Figure (3) presents the changes in uterus weight among the control and experimental groups. The results indicate a significant decrease in uterus weight in the PCOS group ($P \leq 0.01$) compared to the control group. However, treatment with L-arginine or licorice extract led to a significant increase in uterus weight in polycystic rats compared to the untreated PCOS group.

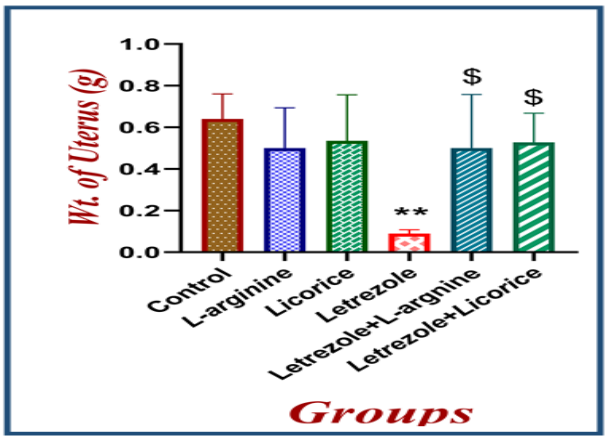

Fig. 3: Uterus weight changes (g) in the control and other experimental groups.

Data illustrated as Mean ± SEM, n = 6

- $^{**}P < 0.01$ compared to control group
- $^{\$}P < 0.05$ compared to PCOS group
- Cont: Control; Letrezole: PCOS group.

3.4. Absolute Ovary weights of the control and other experimental groups

Figure (4) depicts the changes in Ovary Weight across the Control and Experimental Groups.

The results demonstrate a significant increase in ovary weight in the PCOS group ($P \leq 0.01$) compared to the control group. However, treatment with L-arginine or licorice extract resulted in a significant reduction in ovary weight in polycystic rats compared to the untreated PCOS group.

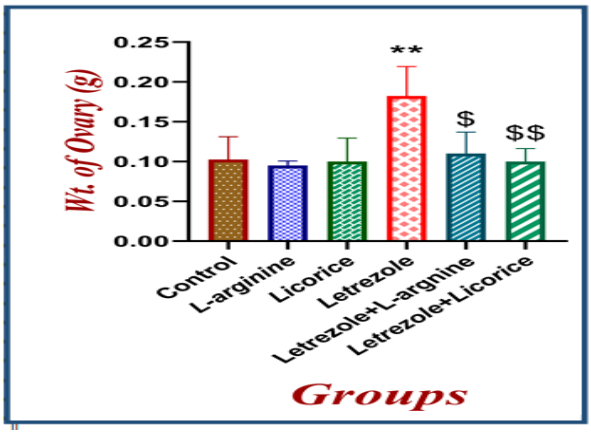

Fig. 4: Ovary weight (g) in the control and other experimental groups.

Data illustrated as Mean ± SEM, n = 6

- $^{**}P < 0.01$ compared to control group
- $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ compared to PCOS group
- Cont: Control; Letrezole: PCOS group.

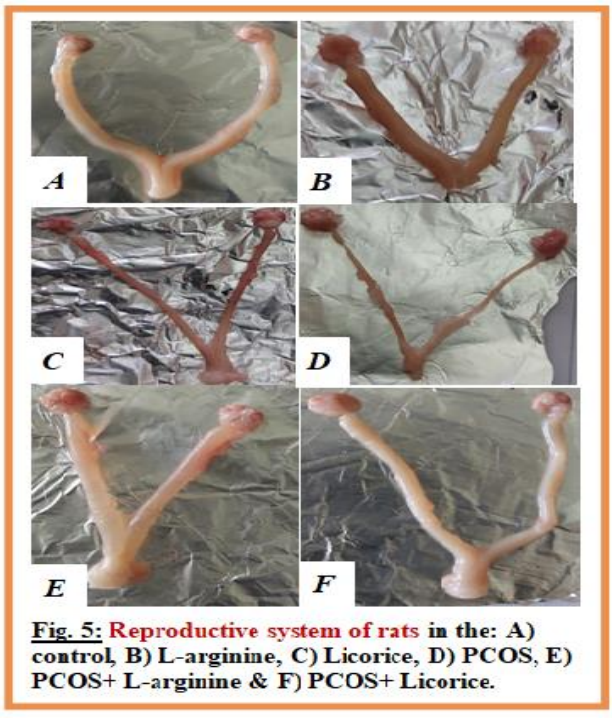

Fig. 5: Reproductive system of rats in the: A) control, B) L-arginine, C) Licorice, D) PCOS, E) PCOS+ L-arginine & F) PCOS+ Licorice.

3.5. Histopathological Changes of Rat Epiphyseal cartilage (X 100) (X 400) in the Control and Different Treated Groups

(A) Control group showed normal histological structure of chondrocyte of the rat epiphyseal cartilage. (B) L-arginine-supplemented group & (C) licorice-supplemented group, revealed normal histological structure of chondrocyte similar to the control group. (D) PCOS group, the epiphyseal cartilage showed multiple histological changes, including an increase in growth plate thickness, aberrant chondrocyte hyperplasia, and a decrease in the quantity of flattened chondrocytes in the superficial zone. (E) PCOS- L-arginine -supplemented group & (F) PCOS- licorice- supplemented group, showed normal histological structure of the rat epiphyseal cartilage similar to the control group. (Fig. 6, 7: A-F) H&E: X 100 & X 400

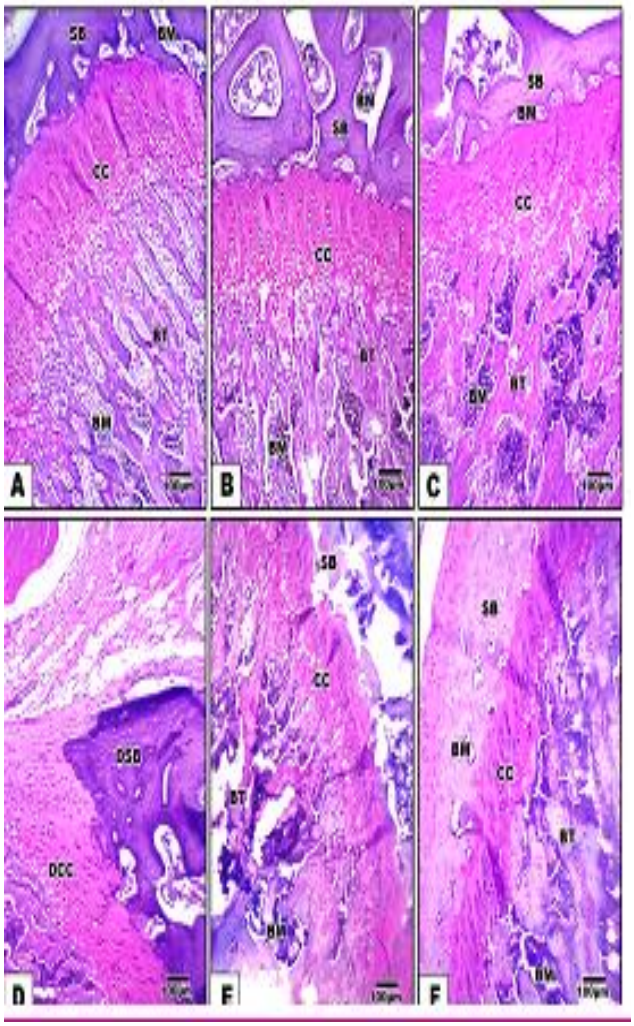

Fig. (6): Photomicrograph of histopathological sections of rat Epiphyseal cartilage in the control and different treated groups stained with Hematoxylin& Eosin, (X 100).

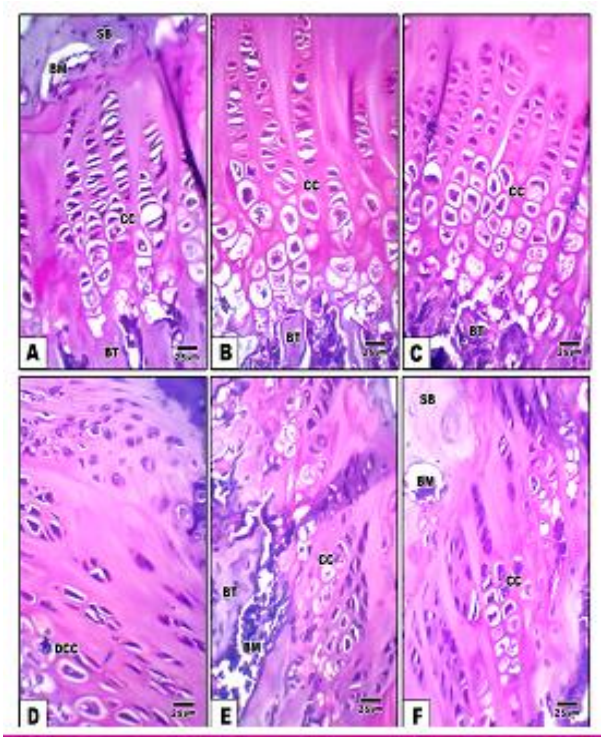

Fig. (7): Photomicrograph of histopathological sections of rat Epiphyseal cartilage in the control and different treated groups stained with Hematoxylin& Eosin, (X 400).

Abbreviations:

SB: Subchondral Bone, BM: Bone Marrow, CC: Calcified Cartilage, BT: Bone Trabeculae, DSB: Developing Subchondral Bone, DCC: Developing Calcified Cartilage.

- In the section of group D, DCC (Developing Calcified Cartilage) is clearly visible, indicating an early stage of calcified cartilage formation. DSB (Developing Subchondral Bone) is also evident, representing an initial phase of subchondral bone development.

- Sections of E and F groups show a more advanced structure with well-formed CC (Calcified Cartilage) and BT (Bone Trabeculae). Also, BM (Bone Marrow) is more prominently visible, reflecting the completion of the differentiation process between bone and cartilage. The bone and cartilage appear more organized compared to group D.

4. Discussion

Polycystic ovarian syndrome (PCOS) is recognized as the most prevalent endocrine disorder affecting women of reproductive age globally. It is diagnosed based on the presence of at least two of the following three criteria: polycystic ovaries, clinical or biochemical hyperandrogenism, and chronic anovulation. Beyond its impact on fertility, PCOS is associated with several significant comorbidities, including obesity, metabolic syndrome, type 2 diabetes mellitus (DM-2), endometrial cancer, and an elevated cardiovascular risk [12].

Recently, there has been growing interest in natural products for managing PCOS and its associated complications. Natural products, such as medicinal plants and fruits, are being investigated as potential PCOS therapies due to their bioactive components, which have pharmacological effects such as antioxidant, antibacterial, anticancer, and antidiabetic qualities. Some of these substances improve insulin sensitivity, reduce inflammation, and boost glucose metabolism, which benefits PCOS patients [13].

One instance is L-arginine, an amino acid that is semi-essential and found in particular in meats and nuts. The enzyme nitric oxide synthase (NOS), which is in charge of producing nitric oxide, uses L-arginine as a substrate [14]. Numerous biological systems, including ovarian physiology, have been shown to benefit from nitric oxide (NO) as an intra- and intercellular modulator [15]. In vivo, either a constitutive calcium-dependent NO synthase or a pro-inflammatory cytokine-inducible NO synthase forms NO from L-arginine [16]. Although its exact function is unknown, follicular maturation and ovulation are two processes that NO is assumed to be involved in [15, 17]. It was proposed [18] that NO might be involved in the control of rat ovarian blood flow during the periovulatory vasodilatatory period.

Another is licorice, a widely used medicinal herb that is thought to be an important and safe medication. Almost 500 different components have been found in licorice roots; the main and most prevalent components are glycyrrhizin and several other flavonoids [19]. Several experimental studies have proven the numerous biological actions of licorice, including its potent antioxidative, antifatigue, antibacterial, antiviral, antiproliferative, and estrogenic properties [20]. Therefore, some studies have suggested licorice extract has been proposed in

some research as a potential treatment for PCOS and infertility [21].

The current investigation found that PCOS significantly increased body weight. In contrast, the polycystic female rats' body weight decreased significantly after receiving L-arginine or licorice extract compared to the PCOS group. This is consistent with other findings by Jobgen, Meininger [22], who reported that L-arginine contributes to body weight reduction. Current evidence supports the idea that physiological levels of arginine and nitric oxide (NO) enhance fat oxidation while reducing fat synthesis in a tissue-specific manner [23]. This is also consistent with the findings of Namazi, Alizadeh [24], who found that licorice lowers body weight. This could be explained by the fact that licorice influences lipid profiles and insulin resistance, two conditions that accompany obesity [25]. Its powerful flavor, which can reduce hunger and food intake, may be the cause of licorice's beneficial effects [26]. The following are some potential ways that licorice reduces obesity: reduction in appetite owing to intense taste [27], decrease in fat intestinal absorption [28], and control of lipid metabolism and lipolysis through effects on gene expression in fatty acid production pathways and increase in fatty acid oxidation [26]. This is also agree with Hooshmandi, Ghadiri-Anari [29], who showed that Licorice supplementation dramatically lowered body weight and BMI levels [30].

In comparison to the control group, the epiphyseal cartilage in PCOS rats exhibited several histological alterations, such as increased growth plate thickness, abnormal chondrocyte hyperplasia, and a reduced number of flattened chondrocytes in the superficial zone. Additionally, the articular cartilage displayed mineralized calcium salt deposits, a decreased cartilage matrix, and superficial fibrillation with erosions. Numerous research, including Mohamed and Yeh [31], found similar outcomes, indicating that long-term use of aromatase inhibitors like letrozole (commonly used to induce PCOS in animal models) has been linked to bone loss and an increased risk of fractures due to estrogen deficiency. Compared to the control group, letrozole administration resulted in significant increases in body weight, bone length, bone area, bone turnover, and resorption activities, but caused a reduction in bone mass and bone mineral density (BMD). Serum estrone and estradiol levels were markedly lower, while testosterone, LH, and IGF-1 levels were significantly elevated. Female PCOS rats treated with letrozole exhibited reduced bone density, increased bone loss, and greater bone separation. The primary mechanism by which letrozole induces BMD loss is believed to involve the suppression of serum estrogen levels.

Fortunately, compared to the PCOS group, the epiphyseal cartilage of the PCOS rats in this study showed improvement after receiving L-arginine or licorice extract. These findings are consistent with those reported by Shen, Wang [32], who observed that L-arginine has been shown to delay osteoporosis in aging mice by supporting bone health. Studies indicate that it enhances mitophagy through PINK1/Parkin and Bnip3 pathways, promotes both osteogenesis and angiogenesis, and reduces adipogenesis. Additionally, it offers protection against oxidative stress (ROS), contributing to the maintenance of bone homeostasis.

5. Conclusion

The study suggests that L-arginine and licorice extract could be potential therapeutic agents for managing PCOS. The findings indicate that oral administration of L-arginine or licorice extract significantly reduces body weight and enhances the structure of epiphyseal cartilage. Additionally, these treatments promote advanced stages of bone and cartilage differentiation and organization in PCOS-induced rats. These results underscore the potential efficacy of these natural compounds in mitigating PCOS-associated symptoms and supporting bone homeostasis.

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