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# Investigating the Effects of Ziziphus Jujuba Extract, Metformin, and Myoinositol on Pregnancy Outcomes and Metabolic Parameters in PCOS Women Undergoing Ovulation Induction: A Randomized Controlled Trial Protocol

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**ABSTRACT**

**Introduction:** Ziziphus jujuba, a flavonoids rich plant and is renowned for its potent antioxidant properties with diverse health benefits across various conditions. This study aims to evaluate the efficacy of Ziziphus jujuba as an adjunct therapy for improving pregnancy outcomes in infertile women diagnosed with normogonadotropic normoestrogenic Polycystic Ovary Syndrome (PCOS) undergoing ovulation induction with letrozole.

**Methods:** A total of 196 participants diagnosed with PCOS and infertility will be recruited from the Milad Infertility Center in Mashhad, Iran. Participants will be randomly assigned to one of four groups: Ziziphus jujuba, Myoinositol, Metformin, or Placebo, with each group consisting of 49 individuals. Over 12 weeks, participants will receive their allocated intervention in conjunction with letrozole for ovulation induction. Clinical and biochemical parameters associated with pregnancy outcomes, including biochemical and clinical pregnancy rates, will be assessed.

**Results:** A total of 196 participants will be included in this study, with 49 participants assigned to each group. It is hypothesized that the Ziziphus jujuba group will exhibit improved glucose metabolism and reduced insulin resistance, as measured by fasting blood glucose (FBG) and triglyceride-glucose (TyG) indices, along with enhanced lipid profiles and reduced inflammatory markers, compared to the other groups. These anticipated metabolic improvements are expected to lead to a higher pregnancy rate in the Ziziphus jujuba group than in the different study groups.

**Conclusion:** This study aims to investigate the potential of Ziziphus jujuba as an adjunctive therapy to improve pregnancy outcomes in infertile women diagnosed with PCOS undergoing letrozole-induced ovulation induction. The findings are expected to provide valuable insights into the role of herbal medicine in addressing fertility challenges associated with PCOS, potentially presenting a cost-effective and accessible alternative to conventional pharmaceutical treatments.



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## Introduction

Infertility is defined as the inability to achieve pregnancy after 12 months of consistent, unprotected sexual intercourse (1). It is a significant global health challenge with profound social, economic, and medical implications (2). Despite its widespread prevalence, the exact global burden of infertility remains uncertain, with estimates ranging from 48.5 million couples worldwide to approximately 186 million ever-married women in developing countries,

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representing about 17.5%, according to recent meta-analyses (3, 4). Among infertility cases, 20–35% are attributed to female factors, with ovulatory dysfunction being the most common cause, characterized by irregular or absent menstrual cycles (5). Polycystic Ovary Syndrome (PCOS) is the leading cause of anovulatory infertility, affecting an estimated 70–80% of women with this condition (6).

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder affecting a significant proportion of the female population. A range of clinical features, including hyperandrogenism, irregular menstrual cycles, and insulin resistance, characterize it (7). The primary approach to managing PCOS involves lifestyle modifications, with a focus on smoking cessation, increased physical activity, and weight loss for those who are overweight or obese (8). Various pharmacological options are available for PCOS treatment, with metformin being widely used due to its ability to improve insulin sensitivity. While studies suggest that metformin may enhance ovulation, its impact on live birth rates remains inconclusive (9-11). Like many medications, metformin is associated with side effects, including gastrointestinal discomfort and, with prolonged use, an increased risk of vitamin B12 deficiency. Its impact on pregnancy outcomes also remains uncertain (12, 13).

In recent years, there has been growing interest in inositol as a potential alternative to metformin for treating PCOS. Classified as part of the vitamin B complex group, inositol functions as an insulin sensitizer, influencing key components of insulin signaling pathways (14). A primary mechanism is its role in facilitating glucose uptake by activating glucose transporter 4 (GLUT4) and reducing fatty acid release from adipose tissue (15). Myoinositol, in particular, helps regulate glucose utilization and uptake. Studies suggest that inositol supplementation may improve ovulation rates and menstrual cycle regularity, although its impact on live birth rates remains uncertain (16). While generally well-tolerated, inositol can cause mild side effects, including digestive discomfort, insomnia, and occasional allergic reactions. Additionally, cost variations may limit accessibility for some individuals (17, 18).

Herbal medicine has become an alternative to conventional pharmaceuticals due to its diverse bioactive compounds with antioxidant, phytoestrogenic, and nutritional properties (19). Among these, jujube (Ziziphus jujuba Mill.) stands out for its significant nutritional value and potential health benefits. Widely cultivated, particularly in China and Iran, jujube has garnered attention for its effects on blood sugar regulation, cholesterol levels, body composition, and antioxidant activity (20-23). Its key bioactive compounds include vitamin C, phenolics, flavonoids, triterpenic acids, and polysaccharides, all contributing to its health-promoting properties (24, 25). Despite its promising benefits, the precise mechanisms by which jujube influences metabolic factors and enhances fertility potential remain unclear, underscoring the need for further research in this area (26, 27).

This study aims to investigate the potential effects of *Ziziphus jujuba* on pregnancy outcomes in women with Polycystic Ovary Syndrome (PCOS). Specifically, the objectives are to i) evaluate the impact of *Ziziphus jujuba* hydroalcoholic extract on biochemical and clinical pregnancy rates, ii) examine its effects on anthropometric indices, blood glucose levels, lipid profiles, and inflammatory markers, and iii) compare its efficacy with metformin and myoinositol in PCOS patients undergoing ovulation induction with letrozole.

## Materials and Methods

This research study is officially registered with the Iranian Registry of Clinical Trials under the code IR.MUMS.MEDICAL.REC.1402.191. Designed as a double-blind, randomized controlled trial, the study will be conducted at the Milad Infertility Treatment Center in Mashhad, Iran. Infertile patients seeking treatment at this facility will undergo an initial evaluation by a gynecologist. Patients diagnosed with Polycystic Ovary Syndrome (PCOS) and recommended for ovulation induction with letrozole will be invited to participate in the study.

The study will include multiple interventions. Initially, all participants will provide informed consent and undergo baseline anthropometric and biochemical assessments. Participants will then be randomly assigned to one of four groups: Ziziphus hydroalcoholic extract, metformin, myoinositol, or placebo. Following a 12-week intervention, blood analyses and anthropometric

measurements will be repeated and compared to baseline values across the groups.

In the subsequent phase, letrozole will be prescribed for ovulation induction, administered on days 3–7 of the menstrual cycle for five days. Participants will use luteinizing hormone (LH) test kits to monitor ovulation. If ovulation occurs, they will be advised to attempt conception. After 14–16 days, a Beta hCG test will be conducted to confirm biochemical pregnancy. For participants with a positive test result, ultrasound sonography will be performed at 6–7 weeks to confirm clinical pregnancy.

To further enhance the efficacy of the study, the research team will include a nutritionist, a midwifery team, and a pharmacologist, who will actively contribute to the research process and its implementation. The study design and procedures are summarized in a flow diagram, presented in Figure 1.

### *Sample size*

Based on the study conducted by Agrawal et al. in 2019, which reported a pregnancy rate of 33.3% in the control group and 63.3% in the intervention group, we utilized a formula for calculating sample sizes in studies with qualitative traits across two populations (28). To achieve a statistical power of 80%, the required sample size for each group was calculated to be 44 participants. Accounting for a 10% allowance for potential dropouts, each group will include 49 participants, resulting in a total study population of 196.

### *Participants selection*

All participants will provide written informed consent before enrollment in the study, facilitated by the research team. The study objectives will be thoroughly explained to each participant, along with detailed guidelines on maintaining a healthy lifestyle and personalized medical interventions throughout the research period.

Inclusion criteria will consist of infertility confirmed by a qualified gynecologist, age between 18 and 45 years, and a diagnosis of Polycystic Ovary Syndrome (PCOS) based on the Rotterdam criteria. Exclusion criteria will apply to participants currently using hormonal medications or those with thyroid dysfunction, liver disease, renal failure, a history of cancer, or who engage in smoking or alcohol consumption before or during the study.

Participants will be withdrawn from the study if they become pregnant within the first 12 weeks of the intervention or fail to comply with their assigned intervention protocol.



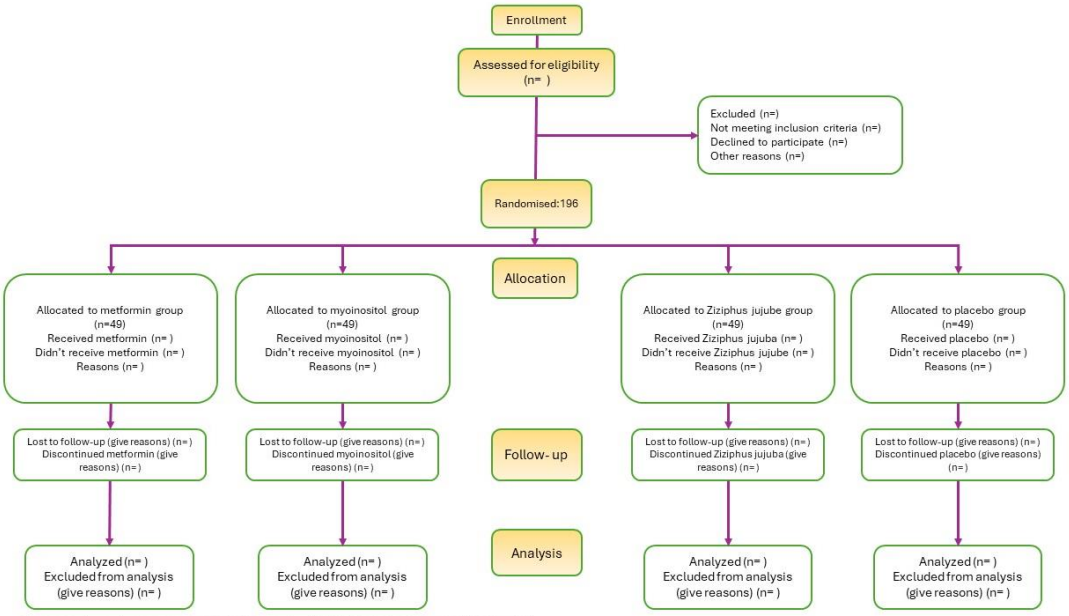


**Figure 1.** Particant flow diagram according to the Consolidated Standards of Reporting Trials (CONSORT)

### *Intervention*

A multifaceted intervention will be implemented, comprising metformin, myoinositol, hydroalcoholic extract of Ziziphus jujuba, and placebo. While metformin and myoinositol are readily available, the Ziziphus jujuba extract will be prepared specifically for the study. Based on the findings of Mostafa et al.'s 2013 study, the recommended effective dose of Ziziphus jujuba is 30 grams per day over 12 weeks (29).

The following steps will be undertaken to prepare the Ziziphus jujuba extract: Jujube fruits will be collected from a garden in Birjand City, Iran, and authenticated at the School of Pharmacy, Mashhad University of Medical Sciences. The fruits will be dried in a dark environment to prevent degradation. A total of 108 kilograms of dried jujube fruits from a single source, validated by botanists and pharmacologists, will be used. A milling machine will grind the dried fruits into a fine powder. A 50% hydroalcoholic extract will be prepared using equal parts of ethanol (96%) and distilled water, ensuring the retention of various flavonoid compounds. The solvent will be removed under low pressure using a rotary evaporator at a controlled temperature of 35°C to preserve the active ingredients of the extract.

The concentrated extract will be blended with Avicel at a 50% ratio to prepare the sachets, converting them into powdered. Each sachet will contain 15 grams of the prepared powder, ensuring a high concentration of *Ziziphus jujube's* active components. Sachets will be prepared monthly to maintain the extract's antioxidant capacity. For standardization, the total flavonoid content of the extract will be quantified using the Specter method, incorporating High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS).

### *Randomization Process*

Following participant selection based on informed consent and the specified inclusion and exclusion criteria, each participant will be randomly assigned to one of the intervention groups. This double-blind, randomized controlled trial is designed to ensure that both participants and investigators remain blinded. To achieve this, the physical appearance of all interventions will be standardized to maintain indistinguishability.

### *Intervention Description*

1. **Group One:** Participants will receive a daily 500 mg metformin pill and a placebo sachet.
2. **Group Two:** Participants will receive a daily myoinositol sachet and a placebo pill.
3. **Group Three:** Participants will receive a daily *Ziziphus jujuba* sachet and a placebo pill.
4. **Group Four:** Participants will receive a daily placebo pill and a placebo sachet.

The randomization process will be conducted using SealedEnvelope.com, an online platform designed to ensure robust and unbiased randomization. An independent statistician, not involved in the study's execution, will generate the randomization schedule using this platform. This independent party will oversee the allocation of participants to intervention groups, which will occur only after the completion of baseline assessments.

To ensure blinding is maintained, unique codes will be assigned to the packaging of each pill and sachet. These codes will be designed to be unrecognizable to both participants and researchers, preventing potential bias or influence on treatment adherence or outcomes. The intervention groups will be designated numerically (Group 1, Group 2, Group 3, and Group 4) to ensure that the specific treatment each participant receives remains concealed throughout the study.

Regular audits ensure strict adherence to the randomization protocol, with any deviations from the assigned groups documented and promptly addressed. This rigorous approach will enhance the trial outcomes' reliability and reinforce the findings' robustness.

Body weight will be measured using a SECA digital scale (Germany) with a precision of 0.1 kg, while height will be measured without shoes using a wall-mounted instrument. Body Mass Index (BMI) will be calculated using the formula: weight (kg) / height ($m^2$).

Biochemical evaluation will involve collecting a 10 mL blood sample from each participant following a 12-hour fasting period. Serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) will be measured using enzymatic colorimetric techniques. The glucose oxidase method will determine fasting blood glucose (FBG) levels. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) will be assessed

through an immunoturbidimetry approach. Insulin resistance will be calculated using the Triglyceride-Glucose Index (TyG), defined as Ln [fasting triglycerides (mg/dL) × fasting plasma glucose (mg/dL) / 2].

### *Ultrasound Evaluation*

Before and after the intervention, all participants will undergo uterine and ovarian sonography performed by a gynecologist using a Philips sonography device with transvaginal affinity at 70 MHz. The sonography will evaluate ovarian morphology to differentiate between Polycystic Ovary Syndrome (PCOS) and normal ovarian morphology.

### *Pregnancy Assessment*

After 12 weeks of intervention, participants will undergo ovulation induction with letrozole at a dosage of 2.5 mg, administered twice daily for 5 days (days 3 to 7 of the menstrual cycle). A follow-up ultrasound will be conducted on the ninth day of the cycle. Participants will be advised to attempt conception if a dominant follicle measuring 18–20 mm is detected. Within 14–16 days, a blood test for beta-hCG will be performed to confirm or rule out pregnancy. Additionally, during the 6th or 7th week of pregnancy, an ultrasound will be conducted to assess the fetal heart rate and confirm clinical pregnancy.

### *Data Collection*

Data will be collected systematically through pre- and post-intervention visits overseen by a multidisciplinary team. Weekly phone calls will monitor participants' well-being and identify potential adverse events. Participants will also undergo monthly evaluations by a fertility specialist to assess their condition and address emerging concerns. If adverse effects become unmanageable, the intervention for that individual will be discontinued. Monthly research team meetings will be conducted to review the study's progress, evaluate patient outcomes, and address any reported side effects. Supplementary data collection tools include a Food Frequency Questionnaire (FFQ) and a Physical Activity Questionnaire, which utilizes the Metabolic Equivalent of Task (MET) concept.

### *Data Management*

Data collection forms will be identified using participant numbers to ensure anonymity. Participants' contact information and the code linking participant numbers to their names will be securely stored in password-protected files. Anonymized data will then be entered into the data repository of Mashhad University of Medical Sciences.

The trial procedure flowchart is presented in Figure 2.



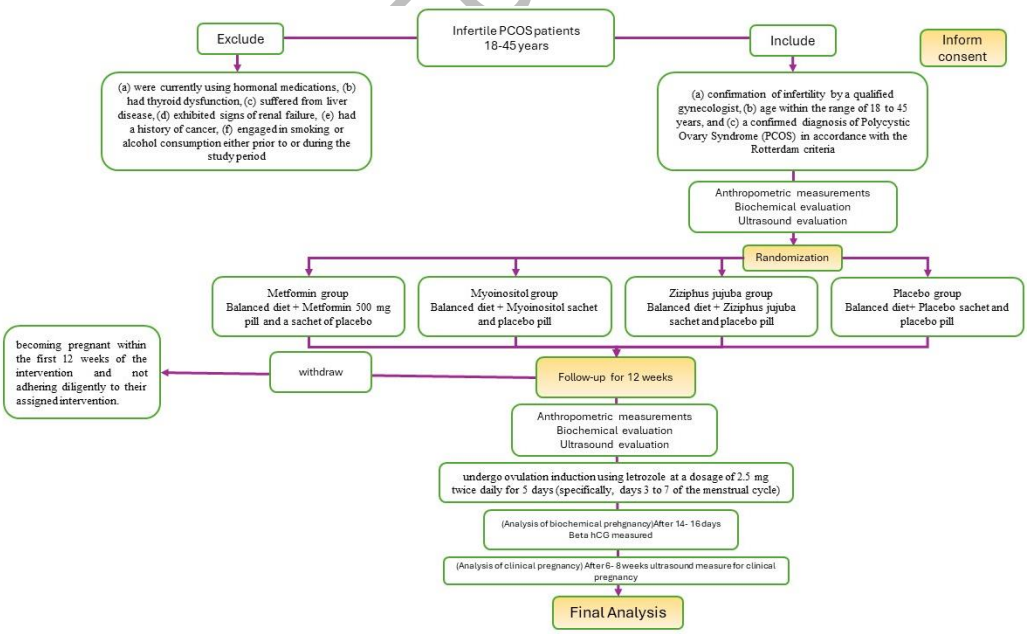


**Figure 2.** Trial procedure flow chat

***Role and Responsibilities***

The research team will comprise specialists in gynecology and infertility, nutritionists, and midwives, working collaboratively to ensure the effective implementation of the trial protocol.

At the initial consultation with the gynecologist, a diagnosis of infertility due to polycystic ovarian syndrome (PCOS) will be confirmed. After providing informed consent, patients will be enrolled in the study protocol and placed under the direct supervision of a gynecologist, nutritionist, and midwife. The midwife will conduct weekly monitoring and will provide ongoing support and assessments. Patients will also receive a contact number for the nutrition team to address any questions or concerns that may arise.

The research team will convene bi-weekly to review the project's progress and resolve any potential issues related to protocol implementation. Additionally, the university's ethics committee will oversee the study by reviewing monthly reports submitted for evaluation.

With the active involvement of the treatment team and regular monitoring, no disruptions to the patient's primary treatment are anticipated. However, if any disturbances or adverse outcomes occur during the study, the research will be paused, and blinding measures will be lifted as necessary.

***Statistical Analysis***

Statistical analysis will be performed using SPSS version 19 (SPSS Inc.), with a significance level set at $P < .05$. Continuous variables will be reported as mean ± standard deviation (SD), and categorical variables will be presented as frequency (%). Normality will be assessed using the Kolmogorov-Smirnov test, as parametric tests require normally distributed data. Non-normal data for non-parametric tests, including the Mann-Whitney U and Wilcoxon signed-rank tests will be employed.

Associations between categorical variables will be evaluated using Chi-squared tests. Independent t-tests compare group means at baseline and follow-up, while paired t-tests assess within-group changes. A covariance (ANCOVA) analysis will be conducted to account for baseline differences and covariates, ensuring precise comparisons of intervention effects.

A biostatistician will oversee the statistical analysis to ensure accuracy and validity. All analyses will be conducted in SPSS version 19, with R software, for advanced checks if necessary.

## Results

This study will include 196 participants, with 49 individuals assigned to each of the four intervention groups. Based on the study design and anticipated outcomes, the *Ziziphus jujuba* group is expected to demonstrate significant improvements in glucose metabolism and insulin resistance, as measured by fasting blood glucose (FBG) levels and the triglyceride-glucose (TyG) index, compared to the other groups.

Additionally, improvements in lipid profiles are anticipated in the *Ziziphus jujuba* group, including reductions in triglycerides (TG) and low-density lipoprotein (LDL) cholesterol, along with increases in high-density lipoprotein (HDL) cholesterol.

Furthermore, markers of inflammation, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), are expected to decrease more significantly in the *Ziziphus jujuba* group compared to the other groups. These changes are hypothesized to result from the metabolic benefits associated with *Ziziphus jujuba*.

## Discussion

This randomized clinical trial aims to investigate the potential effects of Ziziphus jujuba on anthropometric parameters, biochemical factors, and pregnancy outcomes in infertile women with Polycystic Ovary Syndrome (PCOS) undergoing letrozole-induced ovulation. Additionally, a comparative analysis of metformin and myoinositol is included. Previous studies have demonstrated the effectiveness of metformin and myoinositol in reducing insulin resistance and improving the metabolic profile in women with PCOS. However, conflicting evidence exists regarding their impact on pregnancy rates. This study seeks to evaluate the effects of Ziziphus jujuba in these domains and provide new insights into its potential as a therapeutic option (28, 30).

Polycystic Ovary Syndrome (PCOS) is a complex condition characterized by diverse pathophysiological factors that contribute to its heterogeneous clinical manifestations. Among these factors, hyperandrogenism is recognized for its direct and significant role in the development of insulin resistance. Although PCOS is undoubtedly multifactorial, insulin

resistance and elevated androgen levels are considered primary contributors to the syndrome's etiology (31).

Obesity significantly exacerbates the clinical manifestations of PCOS, worsening infertility and reducing the effectiveness of fertility treatments (32). Although the intricate relationship between obesity and PCOS is not fully understood, it represents a critical component of patient management, underscoring the importance of addressing weight loss in this population (33). Furthermore, dyslipidemia is increasingly prevalent in women with PCOS, adding complexity to their clinical profile. As a result, interventions targeting these specific aspects of PCOS—such as obesity and dyslipidemia—offer promising potential for improving outcomes in infertile patients (34).

Lifestyle modifications are the first-line treatment for PCOS, emphasizing their critical role in managing the condition. Metformin and myoinositol are considered secondary therapeutic options. Metformin, a biguanide medication, reduces hepatic glucose production and improves insulin resistance. However, uncertainties persist regarding its efficacy in improving clinical outcomes, alongside concerns about its mild side effects (35). Furthermore, its effects on birth outcomes, fasting glucose levels, serum lipids, and anthropometric parameters remain inconclusive (8).

Inositol, comprising myo-inositol and di-chiro inositol, is an emerging insulin-sensitizing agent with significant efficacy in women with PCOS. Functioning as a second messenger, it is critical in insulin signal transduction. However, previous studies have not demonstrated significant effects on parameters such as BMI, triglycerides, fasting blood glucose, cholesterol levels, or ovulation compared to a placebo (35). Concerns persist regarding its potential risks, including hypoglycemia and suboptimal nutrient absorption. Furthermore, inositol does not appear to have a substantial impact on pregnancy outcomes (8).

*Ziziphus jujuba* has emerged as a promising natural reservoir of nutraceutical and therapeutic compounds (36). Recent studies on jujube fruit's phytochemical and pharmacological properties have highlighted its diverse biological effects. These include improvements in anthropometric indices, immunomodulation, antioxidant activity, antitumor properties, hepatoprotection, hypoglycemic effects, gastrointestinal protection, anticancer potential, anti-inflammatory action, anti-hyperlipidemic and anti-hyperglycemic activities, neuroprotection, sedative effects, and antiviral functions (20, 21, 37). Importantly, recent evidence suggests that *Ziziphus jujuba* can reduce insulin resistance, decrease triglyceride levels, and lower fasting blood glucose, thereby enhancing insulin sensitivity (21-23).

Previous studies have investigated the effects of *Ziziphus jujuba* on insulin resistance, primarily by measuring blood insulin levels. However, these studies did not incorporate the recently introduced Triglyceride-Glucose (TyG) Index, considered a more sensitive and reliable marker of insulin resistance. Our study seeks to evaluate insulin resistance using the TyG index, offering a more comprehensive and accurate assessment of metabolic dysfunction. Moreover, previous research has not explicitly examined the effects of *Ziziphus jujuba* on Polycystic Ovary Syndrome (PCOS), particularly in infertile patients undergoing ovulation induction.

Several mechanisms have been proposed to explain the effects of *Ziziphus jujuba* on various health-related factors. The phenolic compounds in *Ziziphus jujuba*, such as ferulic acid, catechin, and rutin, have been shown to influence glucose metabolism. These compounds exert hypoglycemic effects by inhibiting intestinal α-glycosidase activity, thereby reducing hepatic glucose production and impacting glucose transporters. Additionally, *Ziziphus jujuba* is hypothesized to play a crucial role in regulating glucose and lipid metabolism by activating the adiponectin signaling pathway. Adiponectin is inversely correlated with glucose, triglyceride, very low-density lipoprotein (VLDL), and cholesterol levels while positively associated with high-density lipoprotein (HDL) cholesterol (22, 38, 39).

An alternative mechanism by which *Ziziphus jujuba* may enhance lipid and glucose metabolism involves the preferential utilization of glucose as an energy source over lipids. This process promotes enhanced acetyl-CoA synthesis derived from pyruvic acid, facilitating its entry into the Krebs cycle rather than triglyceride biosynthesis. As a result, the triglyceride-lowering effect of *Ziziphus* extract leads to a significant reduction in very low-

density lipoprotein (VLDL) levels. Since VLDL contributes indirectly to forming low-density lipoprotein (LDL) particles, a significant reduction of VLDL levels is anticipated to correspond with decreased LDL levels. Additionally, given the inverse relationship between plasma high-density lipoprotein (HDL) concentration and plasma triglyceride levels, the triglyceride-reducing effects of *Ziziphus* are expected to result in increased HDL levels. Furthermore, improved glucose metabolism may promote the conversion of proteins into anabolism, enhancing protein synthesis. Apo-A1, a primary structural component of HDL, accounts for approximately 70% of its composition. This suggests that increased HDL levels may be attributed to increased protein anabolism, potentially influenced by *Ziziphus* (40).

*Ziziphus jujuba* contains substantial amounts of pectin, inulin, and unsaturated fatty acids, possibly contributing to its hypocholesterolemic effects. Additionally, its high saponin content positively influences plasma lipid levels. Furthermore, the phytosterols present in *Ziziphus* may inhibit intestinal cholesterol absorption, leading to reductions in both total cholesterol and low-density lipoprotein (LDL) cholesterol (22).

Furthermore, *Ziziphus jujuba* demonstrates significant potential in mitigating inflammation. This fruit is notably rich in vitamin C, which plays a critical role in preventing the production of free radicals. Additionally, ascorbic acid structurally resembles glucose, enabling it to inhibit the non-enzymatic glycosylation of proteins. Studies suggest that *Ziziphus jujuba* may protect against acute and chronic inflammatory responses by inhibiting nitric oxide synthase (NOS). Its effectiveness in reducing oxidative stress is attributed to its abundant natural antioxidant components, including flavonoids, tannins, carotenes, polysaccharide fractions, and vitamins (41).

Previous studies on the effects of *Ziziphus jujuba* on Polycystic Ovary Syndrome (PCOS) have been limited, with only two investigations reported to date. Of these, one study was conducted in animals, while the other involved a very small sample size (42, 43). Furthermore, neither study assessed pregnancy outcomes, focusing solely on clinical symptoms. In contrast, our study is the first to evaluate *Ziziphus jujuba* as a pre-treatment compared to other widely used therapies for PCOS, such as metformin and myoinositol. These treatments are commonly utilized globally for managing PCOS. No previous study has undertaken such a comparative analysis, positioning this investigation as a unique and valuable contribution to the literature.

*Ziziphus jujuba* emerges as a promising herbal pre-treatment option for individuals with Polycystic Ovary Syndrome (PCOS) undergoing ovulation induction. This study is unique in employing the hydroalcoholic extract of *Ziziphus jujuba* as a pre-treatment for infertile PCOS patients undergoing induction ovulation. Additionally, this research represents the first comparative investigation evaluating the outcomes of metformin, myoinositol, and *Ziziphus* extract in this demographic, explicitly focusing on pregnancy outcomes. This pioneering approach highlights the potential benefits and distinctive attributes of *Ziziphus jujuba* in PCOS management, particularly in the context of fertility treatments.

### *Strength and limitations*

Our study possesses several strengths that enhance its credibility. First, including PCOS individuals across the BMI spectrum mitigates selection bias, ensuring a more representative sample. Second, the extended duration and larger sample size contribute to the robustness of the findings. Additionally, the involvement of a multidisciplinary team ensures precision and comprehensive oversight. A notable strength is using *Ziziphus jujuba* extract, which offers a unique and potentially impactful intervention. However, some limitations exist, including variability in product usage and dietary regimens, which may introduce confounders. Nonetheless, patient commitment and regular follow-up are designed to minimize these risks, bolstering the study's internal validity.

### *Trial Status*

The commencement of this clinical trial is scheduled for November 2023, with the hypothesis that data collection will extend through September 2024. [Clinical Trial Number:IRCTID: IRCT20230712058752N1, Registration date: 2023-07-18/ (https://irct.behdasht.gov.ir/trial/71240)]

## Declarations

### *Ethics Considarations and Consent to Participate*

The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (ID: 4020097; IR.MUMS.REC.1402.191). The Human Research Ethics Committee of Mashhad University of Medical Sciences (MUMS) reviewed and approved the study protocol. Written informed consent will be obtained from all participants, with each component of the agreement explained in detail to ensure participants fully understand and agree to the terms of the study.

### *Consent for Publication*

Not applicable.

### *Availability of Data and Materials*

All research data will be securely archived in the data repository of Mashhad University of Medical Sciences. Access to this data by external investigators or industrial entities will be granted upon formal request, subject to approval by the corresponding author and university administration, ensuring the controlled availability of research materials.

### *Conflicts of Interest*

The authors declare no competing interests.

### *Funding*

This study will be funded by Mashhad University of Medical Sciences, Mashhad, Iran (ID:4020097).

### *Authors' Contributions*

M.N. and F.R. conceptualized the study and provided overarching supervision throughout all stages. N.KH. and F.M. was responsible for data collection, while M.KH. designed the methodology. H.R. developed the interventions. All authors actively contributed to the manuscript writing process.

### *Composition of Data Monitoring Committee (DMC)*

Mashhad University of Medical Science constitutes the Data Monitoring Committee (DMC) for this research, ensuring the committee's complete independence from any conflicts of interest.

### *Data Confidentiality*

The personal information of the participants and study data are managed by Mashhad University of Medical Sciences and stored in their secure database to ensure confidentiality. Researchers seeking access to this data must submit a request via email to the research team. The research team will grant access to the requested data upon review and approval.

### *Protocol Amendments*

Significant modifications will be promptly communicated to the corresponding author and, if deemed necessary, will be forwarded to the journal responsible for publishing the article to ensure correction.

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