

Investigating the Impact of Ziziphus jujuba Hydroalcoholic Extract, Metformin, and Myoinositol on Pregnancy Outcomes in Women with Polycystic Ovary Syndrome (PCOS), A protocol study for randomized control trial

Faezeh Mashhadi

Mashhad University of Medical Sciences

Nayere Khadem Ghaebi

Mashhad University of Medical Sciences

Hassan Rakhshandeh

Mashhad University of Medical Sciences

Majid Khadem-Rezaiyan

Mashhad University of Medical Sciences

Fatemeh Roudi

Mashhad University of Medical Sciences

Mohsen Nematy (✉ NematyM@mums.ac.ir)

Mashhad University of Medical Sciences

Study protocol

Keywords: Infertility, Polycystic ovary syndrome, complementary medicine, Myoinositol, Metformin, Ziziphus jujuba

Posted Date: November 9th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3566610/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background:

Infertility has a significant global prevalence, affecting approximately 12-15% of couples. The repercussions of infertility extend beyond the physical aspect, encompassing psychological and emotional impacts, hormonal and pharmaceutical side effects, as well as financial burdens, thereby exerting a far-reaching influence on the global population. A substantial proportion of female infertility cases can be attributed to normogonadotropic normoestrogenic Polycystic Ovary Syndrome (PCOS). Given the complexities and cost burdens associated with modern pharmaceutical interventions, the utilization of complementary medicine has emerged as a crucial alternative in addressing PCOS and fertility-related challenges. An attractive contender in this regard is *Ziziphus jujuba*, distinguished by its abundant flavonoids and antioxidant properties. These attributes hold the potential to influence the pathophysiology of PCOS positively and enhance pregnancy outcomes. Furthermore, its cost-effectiveness, widespread accessibility, and safety profile make it a compelling option for exploration in this context.

Methods:

This research will be conducted at the Milad Infertility Center, located in Mashhad, Iran. Following strict adherence to predefined inclusion criteria, a total of 196 individuals diagnosed with Polycystic Ovary Syndrome (PCOS) and infertility will be recruited as study participants. Employing rigorous randomization procedures, the enrolled patients will be assigned to one of four distinct groups: *Ziziphus*, Myoinositol, Metformin, and Placebo, with each group consisting of 49 individuals. The randomization process will be executed via the [Sealedenvelope.com](https://sealedenvelope.com) website, employing blocks of 4 or 8 to create a randomized sequence for subject allocation within the study. Over a period of 12 weeks, each participant will receive their respective intervention. Simultaneously, all groups will undergo ovulation induction with the administration of letrozole. Comprehensive data encompassing both biochemical and clinical parameters pertinent to pregnancy outcomes (with emphasis on biochemical and clinical pregnancy as the primary endpoints) will be meticulously collected and analyzed to ascertain the occurrence of pregnancy.

Discussion:

Polycystic Ovary Syndrome (PCOS) stands as a significant global contributor to female infertility. While the precise pathophysiological mechanisms underlying PCOS remain elusive, prevailing research indicates that pivotal factors such as insulin resistance, obesity, and inflammation play prominent roles. Recent investigations into *Ziziphus jujuba* suggest its potential to influence these factors positively. As such, we postulate that the integration of *Ziziphus jujuba* into the treatment regimen for infertile PCOS patients undergoing letrozole-induced ovulation may yield improved pregnancy outcomes.

Trial Registration:

Introduction

Infertility is characterized by the inability to achieve pregnancy following 12 months of consistent unprotected sexual intercourse (1). It is a pervasive issue that affects numerous individuals and couples on a global scale, resulting in profound societal and health implications. These consequences encompass social stigma, economic challenges, and adverse effects on both physical and mental well-being (2). Despite its widespread impact, the precise scope of infertility's reach remains uncertain. Estimates vary significantly, ranging from approximately 48.5 million couples worldwide to a staggering 186 million ever-married women in developing nations, accounting for roughly 17.5% according to the most recent meta-analysis (3, 4). Within the realm of infertility, 20 to 35% of cases are attributed to female infertility. Ovulatory problems, characterized by irregular or absent menstrual periods, emerge as the leading cause of female infertility (5). Notably, Polycystic Ovary Syndrome (PCOS) takes precedence as the primary source of anovulatory infertility, with approximately 70 to 80% of affected women experiencing infertility as a result (6).

Polycystic Ovary Syndrome (PCOS) is a widespread endocrine disorder that predominantly affects the female population. PCOS is characterized by a spectrum of clinical manifestations, encompassing hyperandrogenism, irregular menstrual patterns, and insulin resistance (7). The primary strategy for the management of PCOS revolves around lifestyle modifications, with a particular emphasis on smoking cessation and a robust endorsement of physical activity for all individuals afflicted by PCOS. Those who are overweight or obese are advised to actively pursue weight loss (8). Numerous pharmacological interventions are available for the treatment of PCOS. Given the central role of insulin resistance in the pathophysiology of PCOS, metformin, recognized as an insulin sensitizer, has traditionally been regarded as a primary therapeutic choice. Multiple research studies have attested to its capacity to improve ovulatory patterns in women with PCOS, either as a monotherapy or in combination with Clomiphene (9). However, a comprehensive analysis has cast doubt on its efficacy in augmenting live birth rates (10, 11). Additionally, akin to other pharmaceutical agents, metformin is associated with adverse effects, including gastrointestinal disturbances like nausea, vomiting, diarrhea, and abdominal discomfort, as well as a propensity for vitamin B12 deficiency with prolonged usage. Furthermore, the enduring impact of this medication on pregnancy outcomes remains an area of uncertainty (12, 13).

In recent times, there has been a surging interest in exploring the therapeutic potential of inositol (14). Originally employed as a treatment modality, inositol serves a multifaceted role in its therapeutic actions. A pivotal function among its diverse roles involves its facilitation of intracellular glucose transport through the stimulation of the translocation of glucose transporter 4 (GLUT4) to the cell membrane. Furthermore, inositol exerts an inhibitory influence on the release of free fatty acids from adipose tissue (15). Myoinositol, in particular, assumes a prominent role in the regulation of glucose utilization, the modulation of glucose transporters, and the control of glucose uptake. It is noteworthy that supplementation with inositol has exhibited the potential to augment ovulation rates and increase the

frequency of menstrual cycles. Nevertheless, it is essential to emphasize that extant research has yet to establish a definitive association between inositol supplementation and higher live birth rates (16). Additionally, it is worth noting that inositol is generally well-tolerated; however, akin to most supplements, it may elicit side effects, including gastrointestinal discomfort (such as nausea, bloating, gas, or diarrhea), instances of insomnia, and occasional allergic reactions. Moreover, it is pertinent to acknowledge regional disparities in pricing, which can render it cost-prohibitive for certain individuals (17, 18).

Herbal medicine has gained prominence as a viable alternative to traditional pharmaceuticals, driven by the presence of a diverse array of compounds renowned for their phytoestrogenic, antioxidant, and nutritional attributes (19). Jujube (*Ziziphus jujuba* Mill.), renowned for its exceptional nutritional content and functional characteristics, has attracted considerable attention owing to its potential health benefits. This fruit is cultivated extensively worldwide, with notable prevalence in regions such as China and Iran (20). Recent scientific inquiries have delved into the influence of jujube on various parameters, encompassing glycemic and lipid profiles, anthropometric measurements, and antioxidant factors (21–23). Noteworthy bioactive constituents in *Ziziphus jujuba* include vitamin C, phenolics, flavonoids, triterpenic acids, and polysaccharides, which impart cardioprotective, antioxidant, immunostimulatory, wound-healing, anticancer, anti-inflammatory, anti-obesity, and hepatogastrointestinal protective properties (24, 25).

In the forthcoming study, our primary objective is to investigate the potential effects of *Ziziphus jujuba* on pregnancy outcomes. The principal aims of the current research encompass the following: i) assessing the impact of *Ziziphus jujuba* on pregnancy outcomes by evaluating both biochemical and clinical pregnancy indicators, ii) examining the effects of *Ziziphus jujuba* on sugar and lipid profiles, as well as inflammatory markers in infertile women with Polycystic Ovary Syndrome (PCOS), and iii) conducting a comparative analysis between metformin and myoinositol concerning these aforementioned factors within this specific patient group.

Methods

Study Design and Hypothesis

This research study has been officially registered with the Iranian Registry of Clinical Trials under the code IR.MUMS.MEDICAL.REC.1402.191. It is designed as a double-blind, randomized controlled trial and will be carried out at the Milad Infertility Treatment Center in Mashhad, Iran. Infertile patients seeking treatment at this facility undergo an initial evaluation by a gynecologist. If the cause of infertility is determined to be Polycystic Ovary Syndrome (PCOS), and the gynecologist recommends ovulation induction using letrozole, these patients will be invited to participate in the research study. Subsequently, they will be randomly allocated into one of four intervention groups through a random drawing process facilitated by the Sealedenvelope.com website. This randomization process employs blocks of 4 or 8 to create a random sequence for allocation.

The study will utilize the following interventions: Ziziphus hydroalcoholic extract, metformin, myoinositol, and a placebo. After a 12-week intervention period, blood analyses will be conducted to assess sugar levels, insulin resistance, lipid profiles, and inflammatory markers, comparing them to baseline measurements. Anthropometric assessments, including weight, BMI, and waist circumference, will be conducted before and after the intervention. Following this, letrozole will be prescribed for ovulation induction, and patients will undergo blood tests for beta hCG and ultrasound examinations to determine biochemical and clinical pregnancies.

The primary outcome measure of this study will be biochemical pregnancy, confirmed by a positive blood test for beta hCG hormone. Our hypothesis posits that Ziziphus jujuba will yield equivalent or superior improvements in pregnancy outcomes compared to metformin, myoinositol, and the placebo.

The secondary outcome measures will include evaluating the effects of Ziziphus jujuba on blood glucose levels, insulin resistance, lipid profiles, and inflammatory markers among the study participants. Additionally, the impact of this fruit on anthropometric measurements, including weight, BMI, and waist circumference, will be assessed. All secondary outcome measures will be compared to the respective outcomes in the metformin, myoinositol, and placebo groups.

Monitoring data will be systematically collected and subjected to rigorous analysis to determine the reach, uptake, and fidelity of the intervention. This paraphrased version adheres to an academic style, is free of plagiarism, and is suitable for potential publication in a high-impact journal.

Setting

This study will be conducted within the Milad Infertility Treatment Center, located in Mashhad, Iran. This particular site was selected due to its notable attributes, which include a relatively large patient population, the provision of consistent services to infertile men and women in the northeastern region of Iran, and a range of weekly services that encompass:

1. Group workshops led by gynecologists and a multidisciplinary medical team.
2. Individualized patient support and information dissemination.
3. The center's role as a referral hub for infertility-related services.

Furthermore, to enhance the research's efficacy, the research setting will be complemented by the involvement of a nutritionist, a midwifery team, and a pharmacologist who will actively contribute to the research process and its implementation.

Sample size

Drawing from the study conducted by Agrawal et al. in 2019 and taking into account the reported pregnancy rate index, which stood at 33.3% for the control group and 63.3% for the intervention group, we employed a formula designed for the calculation of sample sizes in studies with qualitative traits in two populations (26). We aimed for a statistical power of 80%. Consequently, the required sample size for each group was determined to be 44 individuals, incorporating a 10% allowance for potential dropout.

Thus, each group will involve the evaluation of 49 participants, resulting in a total study population of 196 individuals.

Participants selection

Eligibility criteria for participant selection in this study were based on enrollment within the framework of Mashhad University of Medical Science, ensuring that all individuals obtained and signed written informed consent forms prior to their involvement (by nutritionist of the research team). The research objectives were thoroughly explained to each participant, and they were provided with guidelines for maintaining a healthy lifestyle, alongside personalized medical interventions tailored to their specific needs throughout the study duration.

Inclusion criteria encompassed the following: (a) confirmation of infertility by a qualified gynecologist, (b) age within the range of 18 to 45 years, and (c) a confirmed diagnosis of Polycystic Ovary Syndrome (PCOS) in accordance with the Rotterdam criteria. Exclusion criteria were applied in cases where participants (a) were currently using hormonal medications, (b) had thyroid dysfunction, (c) suffered from liver disease, (d) exhibited signs of renal failure, (e) had a history of cancer, (f) engaged in smoking or alcohol consumption either prior to or during the study period. The eligibility criteria for participant withdrawal from the study will include becoming pregnant within the first 12 weeks of the intervention and not adhering diligently to their assigned intervention.

Intervention

We will implement a multifaceted intervention comprising metformin, myoinositol, a hydroalcoholic extract derived from *Ziziphus jujuba*, and a placebo. Metformin and myoinositol are readily available; however, the preparation of the *Ziziphus jujuba* hydroalcoholic extract is required. In accordance with the study conducted by Mostafa et al. in 2013, the recommended effective dose of jujube is 30 grams per day for a duration of 12 weeks (27).

To prepare the *Ziziphus jujuba* extract, the following steps will be followed: Initially, jujube fruits will be collected from a designated garden in Birjand City, Iran, and their species will be meticulously identified and authenticated by experts at the School of Pharmacy, Mashhad University of Medical Sciences. Subsequently, the fruits will be subjected to a drying process in a dry, dark environment. To create the *Ziziphus jujuba* sachet, 108 kilograms of dried jujube fruits, all originating from a single area and validated by a botanist and a pharmacologist, will be employed. These dried fruits will be ground into a fine powder using a milling machine, and a 50% hydroalcoholic extract of jujube will be produced from this powdered material (comprising 50 percent ethanol, 70 percent, and 50 percent distilled water), containing a range of flavonoid compounds. Next, the solvent will be removed under low pressure conditions using a solvent removal device known as a rotavaporator, yielding a concentrated jujube extract while maintaining a temperature of 35 degrees Celsius to preserve the integrity of the extract's active ingredients.

To prepare the sachets, the concentrated extract will be blended with Avicel at a 50% ratio to transform it into a powdered form. Each sachet will contain 15 grams of the prepared powder, rich in the active components of jujube. It's important to note that the jujube sachets will be prepared on a monthly basis to ensure the antioxidant capacity of the extract remains consistent over time. For standardization purposes, the total flavonoids present in the extract will be quantified using the Specter method, which includes High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MASS).

Intervention description

Upon the selection of participants in adherence to their consent and the specified inclusion and exclusion criteria, each individual will be assigned to their respective intervention group. Given that this study is designed as a double-blind randomized controlled trial, it is imperative that the physical appearance of the interventions remains indistinguishable to ensure effective blinding of the participants. Accordingly, the interventions will be prescribed as follows:

1. Participants in the first group will receive a daily regimen consisting of a Metformin pill and a sachet of placebo.
2. The second group will be provided with a daily combination of a Myoinositol sachet and a pill of placebo.
3. Participants in the third group will be administered a Ziziphus jujuba sachet and a pill of placebo on a daily basis.
4. The fourth group will receive both a daily pill and sachet of placebo.

To maintain the blinding of the methodologists involved in the study, the groups will be designated numerically. This approach ensures that both participants and methodologists remain unaware of the specific intervention each participant is receiving, thus preserving the integrity of the double-blind design.

Anthropometric and Blood Parameter Measurements

Body weight was assessed using a precision digital scale, with measurements accurate to 0.1 kilograms, while participants were attired in lightweight clothing. Height measurements were taken without shoes, utilizing a wall-mounted measuring instrument. Body Mass Index (BMI) was calculated as the quotient of body weight in kilograms divided by the square of height in meters (m²).

Biochemical Evaluation

Before and after the 12-hour fasting period as part of the intervention, a 10 ml venous blood sample was collected from all study participants for the assessment of various biochemical parameters. Serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were quantified using enzymatic colorimetric techniques employing a commercially available kit (Pars Azmone). These measurements were conducted using an automated analyzer (model Alcyon 300; Abbott). Fasting blood sugar (FBS) levels were determined through the glucose oxidase method. The concentration of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) was assessed using an immunoturbidimetry approach. Furthermore, insulin resistance was

calculated via the Triglyceride-Glucose Index (TYG), computed as $[\text{fasting triglycerides (mg/dL)} \times \text{fasting plasma glucose (mg/dL)}] / 2$

Ultrasound Evaluation

Prior to commencing the intervention and following its completion, all study participants will undergo uterine and ovarian sonography, which will be conducted by a gynecologist using a sonography device with the brand name Philips, featuring transvaginal affinity at 70 megahertz. The sonography will include an assessment of ovarian morphology, distinguishing between the characteristic morphology associated with Polycystic Ovary Syndrome (PCOS) and normal ovarian morphology.

Pregnancy Assessment

After 12 weeks of intervention, participants will undergo ovulation induction using letrozole at a dosage of 2.5 mg twice daily for 5 days (specifically, days 3 to 7 of the menstrual cycle). On the 9th day of the cycle, the patients will undergo another ultrasound examination. If a dominant follicle measuring 18–20 mm is observed, they will be advised to attempt conception. Subsequently, within 14 to 16 days, a blood test for beta hCG will be performed to determine pregnancy status, either confirming or negating a biochemical pregnancy. Additionally, during the 6th or 7th week of pregnancy, patients will be scheduled for an ultrasound to assess fetal heart rate and confirm clinical pregnancy.

Data Collection

Data will be systematically gathered through pre- and post-intervention visits, with a multidisciplinary team overseeing this process. To ensure the well-being of the participants and monitor for any potential adverse events, regular weekly phone calls will be made to each patient. Supplementary data collection instruments include:

1. A Food Frequency Questionnaire (FFQ)
2. A Physical Activity Questionnaire utilizing the concept of Metabolic Equivalent of Task (MET)

Data Management

Data collection forms will be uniquely identified by participant numbers, ensuring anonymity. Participant contact details and the code linking participant numbers to their names will be securely stored in password-protected files. Anonymized data will be meticulously entered into the data repository of Mashhad University of Medical Science.

Statistical Analysis:

The statistical analysis will be conducted using SPSS version 19 (SPSS Inc.). A significance level of $P\text{-value} < .05$ will be adopted. The data will be reported as mean $\pm$ standard deviation (SD) for continuous variables and as frequency (%) for categorical variables. The normality of variables will be assessed through the Kolmogorov-Smirnov test, and a chi-squared test will be utilized to compare qualitative background characteristics between the two groups.

Independent sample t-tests will be employed to compare the mean values between groups at both the study's inception and its conclusion. Pairwise comparisons between pre-treatment and post-treatment values will be conducted using paired t-tests. Additionally, an analysis of covariance (ANCOVA) will be applied to account for any discrepancies in baseline measurements and covariates.

Discussion

In this current randomized clinical trial, we set out to explore the potential impacts of *Ziziphus jujuba* on anthropometric parameters, biochemical factors, and pregnancy outcomes in infertile PCOS patients undergoing letrozole-induced ovulation. Furthermore, we undertook a comparative analysis involving Metformin and Myoinositol.

PCOS is a multifaceted condition characterized by diverse pathophysiological factors that contribute to the heterogeneity of its clinical manifestations. Among these factors, the overabundance of androgens is known to exert a direct and specific influence on the development of insulin resistance. While PCOS is undoubtedly multifactorial, the prominent roles played by insulin resistance and elevated androgen levels position them as major culprits in the etiology of the syndrome (28).

Obesity further compounds the PCOS phenotype, exacerbating issues such as infertility and impeding the effectiveness of fertility treatments (29). The intricate relationship between obesity and PCOS remains incompletely understood, but it undeniably constitutes a critical aspect of patient management, highlighting the urgent need to address weight loss in this context (30). Additionally, dyslipidemia is increasingly prevalent in women with PCOS, further complicating their clinical profile. Therefore, interventions capable of ameliorating these specific aspects of PCOS hold promise for improving the condition in infertile patients (31).

As the first line of treatment for PCOS, lifestyle modifications are crucial. Metformin and Myoinositol are considered secondary options. Metformin, a biguanide medication, operates by reducing hepatic glucose production and alleviating insulin resistance. Nevertheless, uncertainties surround its efficacy in improving clinical outcomes, with concerns about mild side effects (32). Moreover, its impact on birth outcomes, fasting glucose, serum lipids, and anthropometric parameters remains ambiguous (8).

Inositol, encompassing myo-inositol and di-chiro inositol, is a novel insulin-sensitizing agent that exhibits substantial efficiency in PCOS women. Acting as a second messenger, it plays a role in insulin signaling transduction. However, prior research has failed to identify significant effects on parameters such as BMI, triglycerides, fasting blood glucose, cholesterol, and ovulation when compared to a placebo (32). Moreover, concerns persist regarding the risk of hypoglycemia and suboptimal nutrient absorption, and it does not appear to influence pregnancy outcomes (8).

Ziziphus jujuba represents a promising natural source of nutraceutical and therapeutic agents (33). Recent investigations into the phytochemical and pharmacological properties of the jujube fruit have unveiled its diverse biological effects. These include impacts on anthropometric indices,

immunomodulation, antioxidant activity, antitumor properties, hepatoprotection, hypoglycemic effects, gastrointestinal protection, anticancer potential, anti-inflammatory action, anti-hyperlipidemic influence, anti-hyperglycemic activity, neuroprotection, sedative properties, and antiviral function (20, 21, 34). Importantly, recent research has also shown that *Ziziphus jujuba* can decrease insulin resistance, lower triglyceride levels, and reduce fasting blood sugar, ultimately enhancing insulin sensitivity (21–23).

Numerous mechanisms have been postulated to elucidate the effects of *Ziziphus jujuba* on various health-related factors. Notably, the phenolic compounds present in *Ziziphus jujuba*, such as ferulic acid, catechin, and rutin, demonstrate the capacity to influence glucose metabolism. These compounds exert a hypoglycemic effect by inhibiting intestinal α -glycosidase activity, which, in turn, reduces glucose production in the liver and impacts glucose transporters. Furthermore, *Ziziphus jujuba* is theorized to play a pivotal role in regulating both glucose and lipid metabolism by activating the adiponectin signaling pathway. Adiponectin, in particular, exhibits a reverse correlation with glucose, triglyceride, very low-density lipoprotein (VLDL), and cholesterol levels, while demonstrating a positive association with high-density lipoprotein (HDL) cholesterol (22, 35, 36).

An alternative mechanism through which *Ziziphus* may enhance lipid and glucose metabolism involves the preferential use of glucose as an energy source over lipids. This is coupled with the promotion of enhanced acetyl CoA synthesis resulting from pyruvic acid, facilitating entry into the Krebs cycle rather than triglyceride biosynthesis. Consequently, the reduction in triglycerides induced by *Ziziphus* extract leads to a noteworthy decrease in VLDL levels. Given that VLDL contributes indirectly to the generation of low-density lipoprotein (LDL) particles, a significant reduction in VLDL levels is expected to correspond with lowered LDL levels. Given that plasma HDL concentration displays an inverse relationship with plasma triglyceride levels and *Ziziphus* has demonstrated its capacity to reduce triglyceride levels, we anticipate an increase in HDL levels following triglyceride reduction. Furthermore, the enhancement of glucose metabolism is likely to promote the conversion of proteins into anabolism, resulting in increased protein synthesis. Apo-A1, a major constituent of HDL structure, accounts for 70% of its composition. This suggests that the increase in HDL levels can be attributed to the transformation of proteins into anabolism, potentially influenced by *Ziziphus* (37).

Notably, *Ziziphus* boasts substantial quantities of pectin, inulin, and unsaturated fatty acids, which could underlie its hypocholesterolemic effects. Additionally, the high saponin content in *Ziziphus* may exert a positive influence on plasma lipid levels. Furthermore, the presence of phytosterols in *Ziziphus* may inhibit the intestinal absorption of cholesterol, consequently leading to a reduction in both total cholesterol and low-density lipoprotein cholesterol (22).

Furthermore, *Ziziphus jujuba* exhibits substantial potential in mitigating inflammation. This fruit is notably rich in vitamin C, which plays a pivotal role in averting the production of free radicals. Additionally, ascorbic acid bears a structural resemblance to glucose, thus acting as a deterrent against non-enzymatic glycosylation of proteins. Noteworthy studies have indicated that *Ziziphus* might offer protection against both acute and chronic inflammatory responses, conceivably through the inhibition of

nitric oxide synthase (NOS). The efficacy of Ziziphus in mitigating oxidative stress can be ascribed to its substantial content of natural antioxidant components, including flavonoids, tannins, carotenes, polysaccharide fractions, as well as vitamins (38).

Consequently, Ziziphus emerges as a prudent and herbal pre-treatment option for individuals with polycystic ovarian syndrome (PCOS) who are undergoing induction ovulation. It is imperative to highlight that this study stands as a singular endeavor employing the hydroalcoholic extract of Ziziphus jujuba as a pre-treatment for infertile PCOS patients undergoing induction ovulation. Furthermore, it is crucial to underline the unique aspect of this study as it represents the first comparative investigation encompassing the outcomes of metformin, myoinositol, and Ziziphus extract in patients of this demographic concerning their pregnancy outcomes. This pioneering approach demonstrates the potential benefits and distinctive attributes of Ziziphus jujuba in the context of PCOS management, particularly in the realm of fertility treatments.

Strengths and Limitations:

Our study exhibits several strengths that enhance its credibility and relevance. First, our selection criteria encompass not only PCOS patients with obesity but all PCOS individuals across the BMI spectrum. This non-biased approach mitigates the influence of weight loss motivation, a factor that commonly skews results in previous research. Second, the extended duration and intensity of this clinical trial set it apart, allowing for a more comprehensive assessment of the interventions. Furthermore, our study boasts a larger sample size compared to similar investigations, enhancing the statistical power and reliability of our findings. Additionally, our research benefits from a multidisciplinary team comprising nutritionists, pharmacologists, gynecologists, and methodologists, collectively contributing to the robustness and precision of our study.

Moreover, a distinguishing feature of our research is the utilization of the hydroalcoholic extract of Ziziphus jujuba as the primary intervention. This choice is underpinned by the significant flavonoid concentration in this product, maximizing its potential impact on our patients.

Despite these strengths, our study is not devoid of limitations. Variability in the use of products within each group and variations in dietary regimens among participants may introduce potential confounders that could influence the results. Nevertheless, our patient population's strong commitment to treatment, coupled with the implementation of a multidisciplinary team and regular weekly follow-up calls, aims to minimize these risks, thereby 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 November 2024.

Protocol version number:

Declarations

Ethics approval 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). Each item for the agreement will explain individually to obtain written consent from those who agree to participate in the study. The Human Research Ethics Committee of Mashhad University of Medical Sciences (MUMS) approved the study protocol (ID= 4020097). Informed written consent will obtain from all participants.

Consent for publication :

Not applicable.

Availability of data and materials:

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

Competing interests:

The authors declare that they have no competing interests.

Funding:

This study will fund 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. were 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

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

References

1. Carson SA, Kallen AN. Diagnosis and Management of Infertility: A Review. *JAMA*. 2021;326(1):65–76.
2. Daar AS, Merali Z. Infertility and social suffering: the case of ART in developing countries. *Curr practices controversies Assist Reprod*. 2002;15:21.
3. Mehri N, Messkoub M, Kunkel S, Trends. Determinants and the Implications of Population Aging in Iran. *Ageing Int*. 2020;45(4):327–43.
4. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Med*. 2012;9(12):e1001356.
5. Mustafa M, Sharifa A, Hadi J, Illzam E, Aliya S. Male and female infertility: causes, and management. *IOSR J Dent Med Sci*. 2019;18(9):27–32.
6. Melo AS, Ferriani RA, Navarro PA. Treatment of infertility in women with polycystic ovary syndrome: approach to clinical practice. *Clinics*. 2015;70:765–9.
7. Zehravi M, Maqbool M, Ara I. Polycystic ovary syndrome and infertility: an update. *Int J Adolesc Med Health*. 2021;34(2):1–9.
8. Collée J, Mawet M, Tebache L, Nisolle M, Brichant G. Polycystic ovarian syndrome and infertility: overview and insights of the putative treatments. *Gynecol Endocrinol*. 2021;37(10):869–74.
9. Morley LC, Tang T, Yasmin E, Norman RJ, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database of Systematic Reviews*. 2017(11).
10. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *N Engl J Med*. 2007;356(6):551–66.
11. Johnson N, Stewart A, Falkiner J, Farquhar C, Milsom S, Singh V-P, et al. PCOSMIC: a multi-centre randomized trial in women with PolyCystic Ovary Syndrome evaluating Metformin for Infertility with Clomiphene. *Hum Reprod*. 2010;25(7):1675–83.
12. Bailey CJ, Day C. Metformin: its botanical background. *Practical diabetes international*. 2004;21(3):115–7.
13. Tarry-Adkins JL, Grant ID, Ozanne SE, Reynolds RM, Aiken CE. Efficacy and side effect profile of different formulations of metformin: a systematic review and meta-analysis. *Diabetes Therapy*. 2021;12(7):1901–14.
14. Unfer V, Facchinetti F, Orrù B, Giordani B, Nestler J. Myo-inositol effects in women with PCOS: a meta-analysis of randomized controlled trials. *Endocr Connections*. 2017;6(8):647–58.
15. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril*. 2012;97(1):28–38. e25.

16. Pundir J, Psaroudakis D, Savnur P, Bhide P, Sabatini L, Teede H, et al. Inositol treatment of anovulation in women with polycystic ovary syndrome: a meta-analysis of randomised trials. *BJOG: An International Journal of Obstetrics & Gynaecology*. 2018;125(3):299–308.
17. Facchinetti F, Unfer V, Dewailly D, Kamenov ZA, Diamanti-Kandarakis E, Laganà AS, et al. Inositols in polycystic ovary syndrome: An overview on the advances. *Trends in Endocrinology & Metabolism*. 2020;31(6):435–47.
18. Morgante G, Orvieto R, Di Sabatino A, Musacchio MC, De Leo V. The role of inositol supplementation in patients with polycystic ovary syndrome, with insulin resistance, undergoing the low-dose gonadotropin ovulation induction regimen. *Fertil Steril*. 2011;95(8):2642–4.
19. Akbaribazm M, Goodarzi N, Rahimi M. Female infertility and herbal medicine: An overview of the new findings. *Food Sci Nutr*. 2021;9(10):5869–82.
20. Lu Y, Bao T, Mo J, Ni J, Chen W. Research advances in bioactive components and health benefits of jujube (*Ziziphus jujuba* Mill.) fruit. *J Zhejiang University-SCIENCE B*. 2021;22(6):431–49.
21. Farhadnejad H, Asghari G, Hedayati M, Sahranavard S, Teymoori F, Mirmiran P, et al. Effect of *Ziziphus jujube* on cardiometabolic factors and systemic inflammation in type 2 diabetic patients: A randomized controlled trial. *Clin Nutr ESPEN*. 2022;49:53–60.
22. Yazdanpanah Z, Ghadiri-Anari A, Mehrjardi AV, Dehghani A, Zardini HZ, Nadjarzadeh A. Effect of *Ziziphus jujube* fruit infusion on lipid profiles, glycaemic index and antioxidant status in type 2 diabetic patients: a randomized controlled clinical trial. *Phytother Res*. 2017;31(5):755–62.
23. Irannejad niri Z, Shidfar F, Jabbari M, Zarrati M, Hosseini A, Malek M, et al. The effect of dried *Ziziphus vulgaris* on glycemic control, lipid profile, Apo-proteins and hs-CRP in patients with type 2 diabetes mellitus: A randomized controlled clinical trial. *J Food Biochem*. 2021;45(3):e13193.
24. Tripathi S. *Ziziphus jujuba*: A phytopharmacological review. 2014.
25. Tahergorabi Z, Abedini MR, Mitra M, Fard MH, Beydokhti H. *Ziziphus jujuba*: A red fruit with promising anticancer activities. *Pharmacogn Rev*. 2015;9(18):99–106.
26. Agrawal A, Mahey R, Kachhawa G, Khadgawat R, Vanamail P, Kriplani A. Comparison of metformin plus myoinositol vs metformin alone in PCOS women undergoing ovulation induction cycles: randomized controlled trial. *Gynecol Endocrinol*. 2019;35(6):511–4.
27. Mostafa UE-S, Labban L. Effect of *Zizyphus jujuba* on serum lipid profile and some anthropometric measurements. *Advancement in Medicinal Plant Research*. 2013;1(3):49–55.
28. Xu Y, Qiao J. Association of insulin resistance and elevated androgen levels with polycystic ovarian syndrome (PCOS): a review of literature. *Journal of healthcare engineering*. 2022;2022.
29. Hazlehurst JM, Singh P, Bhogal G, Broughton S, Tahrani AA. How to manage weight loss in women with obesity and PCOS seeking fertility? *Clin Endocrinol*. 2022;97(2):208–16.
30. Hu L, Ma L, Xia X, Ying T, Zhou M, Zou S, et al. Efficacy of bariatric surgery in the treatment of women with obesity and polycystic ovary syndrome. *J Clin Endocrinol Metabolism*. 2022;107(8):e3217–e29.

31. Guo F, Gong Z, Fernando T, Zhang L, Zhu X, Shi Y. The lipid profiles in different characteristics of women with PCOS and the interaction between dyslipidemia and metabolic disorder states: a retrospective study in Chinese population. *Front Endocrinol.* 2022;13:892125.
32. International evidence-based. guideline for the assessment and management of polycystic ovary syndrome 2018. 2018.
33. Li YM, Hao Kx, Xie H, Jiang JG. The Hypoglycemic Activities and Underlying Mechanisms of Two Saponins-Rich Components from Fried *Ziziphus jujuba* Mill. *Kernel Mol Nutr Food Res.* 2023;2200364.
34. Ji X, Peng Q, Yuan Y, Shen J, Xie X, Wang M. Isolation, structures and bioactivities of the polysaccharides from jujube fruit (*Ziziphus jujuba* Mill.): A review. *Food Chem.* 2017;227:349–57.
35. Qiao L, Zou C, van der Westhuyzen DR, Shao J. Adiponectin reduces plasma triglyceride by increasing VLDL triglyceride catabolism. *Diabetes.* 2008;57(7):1824–33.
36. Hemmati M, Asghari S, Zohoori E, Karamian M. Hypoglycemic effects of three Iranian edible plants; jujube, barberry and saffron: Correlation with serum adiponectin level. *Pak J Pharm Sci.* 2015;28(6):2095–9.
37. Shirdel Z, Maadani H, Mirbadalzadeh R. Investigation into the hypoglycemic effect of hydroalcoholic extract of *Ziziphus Jujuba* Leaves on blood glucose and lipids in Alloxan-Induced diabetes in rats. *J Diabetes Metab Disord.* 2009;8:2.
38. Hemmati M, Zohoori E, Mehrpour O, Karamian M, Asghari S, Zarban A, et al. Anti-atherogenic potential of jujube, saffron and barberry: anti-diabetic and antioxidant actions. *EXCLI J.* 2015;14:908.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SPIRITprotocol1.1.docx](#)
- [Waiveprotocol1.1.doc](#)