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Oral intake of *Salvia officinalis* improves intrauterine insemination outcomes in women with polycystic ovary syndrome

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SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection. **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

Background: Polycystic Ovary Syndrome (PCOS) is a common cause of infertility. The usual management of PCOS is Ovulation Induction (OI) before trying assisted reproductive technologies. A being safe treatment, Intrauterine Insemination (IUI) could benefit PCOS patients' fertility.

Objectives: The current research was aimed to evaluate the effect of Salvia officinalis extract on PCOS patients undergoing IUI.

Methods: Four hundred sixty- one PCOS women diagnosed according to Rotterdam criteria attended the Infertility Clinic of The High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University/ Baghdad/ Iraq, during the period from June 2020 to June 2024. The patients were grouped randomly into two groups (Group A) received 500 mg of oral Salvia officinalis leaf extract capsules for 8 weeks before IUI; (Group B) received placebo capsules for the same duration. Demographic parameters, baseline hormonal levels, ovarian stimulation characteristics, and pregnancy outcomes were analyzed and compared between the two groups. A total of 400 IUI cycles were included, 200 in each group.

Results: The statistical analysis showed no significant difference in demographic parameters or baseline hormonal levels between the groups. Regarding ovarian stimulation characteristics; number of dominant follicles, endometrial thickness, serum E2, were significantly higher in group A (p= 0.003, p= 0.008, p= 0.002) respectively. The clinical pregnancy rate, live birth rate of group A were significantly higher (p= 0.0274, p= 0.0274).

Conclusion: Salvia Officinalis extract can improve the clinical pregnancy rate, and live birth rate among an ovulatory PCOS patients undergoing IUI.

Keywords: IUI; Natural products; PCOS; Pregnancy outcomes; Salvia officinalis

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common cause of anovulatory infertility worldwide [1]. It may contribute to 80% of cases, and affecting about 9.2% of reproductive-aged women [2]. Anovulation in women with PCOS is due to arrest of antral follicle development prior to the mature, pre-ovulatory stage [3,4]. Hyperandrogenism and insulin resistance are one of the fundamental features of PCOS [5,6]. These 2 factors enhance oxidative stress [7-10]. Many other factors may participate in production of ROS in PCOS women, as many researchers proved an increased oxidant status in non-obese PCOS women without insulin resistance [11-14]. The oxidative stress may develop as a result of misbalance between the production of free radicals and antioxidant resulting negative equilibrium shift causing disruption of mitochondrial DNA (mtDNA), and proteins damage inducing cell apoptosis [15-20]. A vast number of reports indicate that women with PCOS experience a persistent oxidative stress asymmetry, and OS has emerged as a crucial component in the development of PCOS [21,22]. Normal reactive oxygen species (ROS) is crucial for normal folliculogenesis, and oocyte maturation [23-26].

Medicinal plants and their bioactive constituents have been used for decades by humans as a source of natural therapies in treating different health problems [27-29]. Salvia Officinalis is an everlasting rounded small tree in the Labiatae/Lamiaceae family. That is common in Mediterranean and the Middle East [30]. Salvia Officinalis is widely used in cooking and pharmaceutical industries [31-33]. The biological effect of Salvia Officinalis has been studied by many researchers, which revealed, antioxidant, anti-inflammatory, hypoglycemic, and hypolipidemic properties [34,35]. Several studies support, nutritional supplements usage as adjuvant therapy in treating PCOS [36]

The intrauterine insemination (IUI) is types of assisted reproduction technologies used worldwide, because it is relatively simple, need shorter time for patients' preparation, lower cost than IVF/ICSI, with a very low prevalence of complications [37-40].

Many Authors recommended IUI as 1st line treatment before switched to IVF/ICSI, which might fasten pregnancy for target women with less invasive measures. Intrauterine insemination (IUI) has been considered as common in infertility treatment. It is frequently used in couples with mild male factor infertility, unexplained infertility, mild endometriosis, cervical factors and psychological sexual dysfunction [22,41-44]. Therefore, the current study aimed to determine whether Salvia Officinalis intake can improve IUI outcomes in PCOS patients.

PATIENTS AND METHODS

Study design and setting: This prospective, randomized double -blinded clinical trial was done at the Infertility Clinic of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University/ Baghdad/ Iraq, during the period from June 2020 to June 2024. The study approved by the Ethical Committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies.

Treatment protocol: The patients were grouped into two groups; Group (A) received 500 mg of oral capsules of Salvia Officinalis for 8 weeks before IUI, Group (B) received placebo for the same duration. All the participants started ovulation induction at the third day of the cycle after evaluation of antral follicular count (AFC), and exclusion of ovarian cyst by transvaginal sonography. The enrolled patients received letrozole (Denk pharma) 2.5 mg twice daily for 5 days. On the day eight of the cycle, 75 units of recombinant purified FSH (Gonal-F, Merck Serono, Switzerland) were given according to individual patient responses. In all treatment cycles, continuous monitoring of the follicular size every 2 to 3 days. When the follicle diameter reached ≥ 18 mm, and the number of follicles measuring≥14 not more than 3 triggering of ovulation was done by 6500 IU units of recombinant HCG (Ovitrell, Merck Serono, Switzerland) the serum estradiol level, and endometrial thickness assessed for all patients. Intrauterine insemination was done 36 hours later

Semen preparation and IUI procedure: An ultrasound examination was done to confirm clinical pregnancy, multiple pregnancy and fetal number, and viability 2-3 weeks after positive chemical pregnancy test (> 10 mIU/ mL). Findings of more than one gestational sac in the uterus, was defined as multiple pregnancy. All pregnant patients had followed-up. Pregnancy loss before 12 weeks of gestation defined as early miscarriage [45-47]. Giving birth y of a living fetus (or fetuses) beyond 28 weeks of gestation defined as Live birth [48].

Pregnancy outcome evaluation: An ultrasound examination was done to confirm clinical pregnancy, multiple pregnancy and fetal number, and viability 2-3 weeks after positive chemical pregnancy test (>10 mIU/ mL). Findings of more than one gestational sac in the uterus, was defined as multiple pregnancy. All pregnant patients had followed-up. Pregnancy loss before 12 weeks of gestation defined as early miscarriage [40-42]. Giving birth y of a living fetus (or fetuses) beyond 28 weeks of gestation defined as Live birth [43].

Ethical consideration and registration details

This research was conducted in accordance with the Declaration of Helsinki. Because our country lacks its own primary registries, clinical trial numbers are not applicable. However, this study was registered by the local IRB at the high institute for infertility diagnosis and assisted reproductive technologies, Al-Nahrain University under permission code number 0701-PF-2024M41. Patients were given a concise explanation of the study's objectives and methodologies.

Statistical analysis

All of the statistical analyses were done using statistical package SPSS-28 (IBM Corporation, USA). Qualitative variables were expressed as number and percentage. To compare the proportions, fisher exact test was used. For quantitative variables Mean ± SD was used. Student's t test was used for comparison of quantitative variables between the groups. Statistical significance was considered at P value of < 0.05.

RESULTS

A total of four Hundred sixty- one couples underwent their 1st IUI cycle were participated in this study. Sixty-one couples were excluded because unfitting the inclusion criteria or because of poor patients' compliance.

Comparison of patient characteristics

Demographic parameters and baseline hormones of both groups were compared. There was no significant difference (p>0.05) in males' average age, females' average age, BMI, duration, and type of infertility as shown in **Tab. 1**.

Regarding baseline hormonal levels there were no significant difference in basal levels of FSH, LH, E2, and progesterone as shown in **Tab. 2**.

IUI characteristics

Comparison of the IUI cycle characteristics between both Groups illustrated that the total number of dominant follicles, endometrial thickness, and Estradiol level at day of triggering were significantly higher (p<0.05) in Group (A) as shown in **Tab. 3**.

The pregnancy outcomes

The clinical pregnancy rates of both groups was 26% and 16.5 % respectively, which was significantly higher in Group A (p=0.027) live birth rate of both groups were 25.5%, 16.5% respectively; and it was significantly higher in Group A (p=0.027). However, there were no significant differences in early miscarriage rate, multiple pregnancy rate. There were no cases of ovarian hyperstimulation, or ectopic pregnancy in this study. As shown in **Tab. 4.**

DISCUSSION

Due to the complexity of the pathogenesis of Polycystic ovary syndrome, many nutritional supplements had been tried in addition to conventional medications in management of this disorder assuming their beneficial effects in improving metabolic, and endocrine disorders [49]. The results of the present study were comparable between the two groups regarding males' and females' age, BMI, type and duration of infertility (Tab. 1.) in addition to comparable basal hormonal levels (Tab. 2.). These findings were important to be sure about statistical matching and to reduce any variations that could affect the study's outcome. This study recorded better findings regarding the number of dominant follicles in group treated with Salvia Officinalis. This finding was consistent with an earlier investigation by Alrezaki et al., who observed that mRNA levels of different genes responsible for a dramatic increase in folliculogenesis and steroidogenesis with a high dose of Salvia Officinalis [50], As a result, a significant increase in the number of growing follicles was found, whereas the number of

Tab. 1. A comparison of demographic characteristics between the studied groups.

Demographic Characteristics	Group (A)	Group (B)	P- value
Female age/years	30.8 ± 1.16	29.2 ± 1.7	0.08 NS
Male age	32 ± 3.84	31.4 ± 2.15	0.396 NS
Type of infertility			
Primary	n=91 (45.5%)	n=86 (43%)	0.687 NS
Secondary	109 (54.5%)	114 (57%)	
Duration of infertility/ years	3.5 ± 0.44	3.3 ± 1.16	0.370 NS
Female BMI (kg/m$^{2)}$	26.2 ± 1.83	26 ± 1.41	0.433 NS

Data presented as Mean ± SD
NS= no significant.
Number of patients without group A= 200; Number of patients group B=200
BMI= Body Mass Index difference
Non parametric T Test (Mann-Whitney Test); for type of infertility Chi Square test used.

Tab. 2. Comparison of basal hormones levels between studied groups.

Baseline sex hormone	Group (A)	Group (B)	P- value
FSH (mIU/mL)	3.54 ± 0.53	4.38 ± 0.87	0.600 NS
LH (IU/mL)	6.64 ± 1.03	7.24 ± 1.178	0.208 NS
CD2 E2 (pg/ mL)	48.6 ± 2.4	46.4 ± 4.39	0.17 NS
Progesterone (ng/ mL)	1.28 ± 0.13	1.28 ± 0.13	0.5 NS

Data presented as Mean ± SD
Number of patients in group A= 200; Number of patients in group B= 200
FSH= Follicular Stimulation Hormone, LH=Luteinizing Hormone, E2= Estradiol, CD= cycle day
NS= no significant difference p>0.05
* Non parametric T Test (Mann-Whitney Test).

Tab. 3. A comparison of the IUI cycle characteristics between studied groups.

Characteristics	Group (A)	Group (B)	p- value
Number of DF	2.8 ± 0.447	1.8 ± 0.447	0.003*
ET	9.24 ± 0.0502	8.08 ± 0.708	0.008*
E2 at day of trigger (pg/ mL)	430 ± 59.05	290 ± 56.5	0.002*

Data presented as Mean ± SD
* Significant difference p<0.05
Number of patients in group A= 200; Number of patients in group B= 200
DF= dominant follicles, ET= endometrial thickness, E2= Estradiol
Non parametric T Test (Mann-Whitney Test).

Tab. 4. Comparison of the IUI cycle outcome between studied groups.

IUI Outcome	Group (A)	Group (B)	P- value
Clinical pregnancy rate	52/200 (26%)	33/200 (16%)	0.0274*
Early miscarriage	1/52 (1.8%)	2/33 (6.06%)	0.557 NS
Live birth	51/200 (25.5%)	33/200 (16.5%)	0.0274*
Multiple pregnancy	3/52 (5.76%)	2/33 (6.06%)	0.55 NS

Number of patients in group A= 200; Number of patients in group B= 200
* Chi-Square Tests
S= significant difference p<0.05
NS= no significant difference p>0.05

abnormal follicles was decreased. Other research also revealed that Salvia Officinalis promoted growth [51].

Endometrial thickness was more in group received Salvia Officinalis capsule and this may be due to higher number of growing and matured Graafian follicules, besides a number of corpora lutea and improve fertility [52]. Estradiol level at the day of ovulation triggering in patients who received Salvia Officinalis may be attributed to its effect in decreasing body mass index and in decreasing insulin resistance as found by Leila Amini et al.[53] and can be explained by the phytoestrogenic effect of Salvia Officinalis as proved by Ghorbani and Esmaeilizadeh [33]. Additionally, estradiol level was higher in group (A) and this is important because IUI success is multifactorial, maturing multiple follicles could not merely predict cycle success. However, Estradiol level at the day of trigger affected pregnancy rate [39]. As phytoestrogens shown to be effective treatment for PCOS syndrome symptoms by reducing androgen levels, through inhibiting the activities of 3β-HSD and/or 17β-HSD enzymes, and reduction of oxidative stress levels [54]. Many studies on animal models approved that Salvia Officinalis extract enhances ovarian function by inducing steroid hormones production and folliculogenesis [50].

STUDY LIMITATIONS

The difficulties faced the study were the limited number of cases and missing of a lot of cases during treatment period.

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ETHICAL APPROVAL

This research was conducted was conducted in accordance with the Declaration of Helsinki. This study was approved by the ethical committee of high institute for infertility diagnosis and assisted reproductive technologies, Al- Nahrain University, Baghdad, Iraq after evaluating the most recent instalment, topic information, and research plan on June 6, 2020, as per approval code number 0701-PF-2024M41. An informed written/ oral consent was obtained from all participants.

AUTHOR CONTRIBUTION

LAA prepared the final copy of the manuscript and participated in funded supplies, equipment, and resources of the research centre. MAJ developed and planned the study idea, acquired data, organized materials and contributed to the rewritten paperwork and statistical data calculation. MSA researched and harvested the information that broadened the printed document, integrated the methodological choice, and take part in electronic reinforcement. AAMK aided with the presenting of data, supervision, comprehension, gathering, and accurate assessment of the materials and methods employed during this study program. HRS developed the study-project's conceptual basis, partnered with others to proofread the documentation and provided logistic support.

DATA AVAILABILITY

The data will be made available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

There are no conflict of interest interests to declare.

FUNDING

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