



# Celery Plus Anise Versus Metformin for Treatment of Oligomenorrhea in Polycystic Ovary Syndrome: A Triple-Blind, Randomized Clinical Trial

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## Abstract

**Background:** Polycystic ovary syndrome (PCOS)-induced oligomenorrhea can leave psychological and non-psychological impacts on women. Among the proposed strategies for its treatment, herbal medications are of importance due to favorable effect profiles.

**Objectives:** We planned a study to compare the effects of Celery and Anise combination (CAC) and metformin (met.) on oligomenorrhea in PCOS patients.

**Methods:** We conducted a triple-blind, Randomized clinical trial on 72 patients that were randomly allocated into two equal groups to receive six capsules of either CAC (750 mg each) or met. (250 mg each) at three separate doses for 15 days beginning from the follicular phase. If the bleeding occurred, three capsules of either placebo or met. would be administered daily during the menstruation phase, and then the follicular phase step with six capsules would be repeated. If the bleeding did not occur, three capsules of either placebo or met. would be administered each day for 15 days. The regularity of menstrual bleeding as the primary outcome, as well as testosterone, luteinizing hormone/follicular stimulating hormone ratio (LH/FSH), and complications, was assessed before and after the three cycles.

**Results:** The mean age  $\pm$  standard deviation of patients was  $26.5 \pm 6.1$ . The mean Body Mass Index was  $26.4 \pm 3.5$ . CAC significantly improved oligomenorrhea (58.3% vs. 25%,  $P < 0.01$ ), increased bleeding episodes ( $P = 0.003$ ), and reduced testosterone (mean difference: 0.16 vs. -0.02,  $P = 0.005$ ) and LH/FSH (mean difference: 0.75 vs. -0.08,  $P = 0.002$ ) without any major side effects compared to met.

**Conclusions:** We showed that Celery and Anise combination could regulate menstrual cycles and improve oligomenorrhea in polycystic ovary syndrome patients superiorly to metformin.

**Keywords:** Medicine, Metformin, Oligomenorrhea, Polycystic Ovary Syndrome, Traditional, *Apium graveolens*, *Pimpinella anisum*

## 1. Background

Polycystic ovary syndrome (PCOS) is a common and complicated disorder involving female endocrine, reproductive, and metabolic systems, affecting 2.2% - 19.9% women at reproductive age (1-3). According to the Rotterdam's criteria, PCOS prevalence reaches as high as 14.6% in Iran (3).

Although the exact etiology of PCOS has not been fully clarified, it is thought to be a multifactorial disorder involving extremist steroidogenesis in ovaries, insulin re-

sistance, oxidative stress, and genetic and environmental factors (2, 4). It is commonly manifested by oligomenorrhea/amenorrhea, clinical or biochemical hyperandrogenism, and polycystic ovaries (5). Menstrual irregularities as oligomenorrhea are observed in nearly 70% of the women with PCOS (6). Treatment strategies are based on the manifestations of PCOS. In addition, treatment of oligomenorrhea, which frequently happens during PCOS, is crucial due to its adverse effects on the cardiovascular system, as well as ovarian function, endometrial and ovar-

ian cancers, and psychological disorders (4, 5). Combined oral contraceptive pills are recommended for the treatment of menstruation-related disorders in PCOS women (5). Evidence suggests that insulin-lowering agents such as metformin (met.) may also improve PCOS-induced oligomenorrhea and anovulation and decrease insulin resistance (1, 5). Due to the inadequate understanding of the exact causes of PCOS and its reproductive, metabolic, and cardiovascular complications, as well as the several side effects of hormonal products, the use of other therapeutic strategies such as complementary and alternative medicine (CAM), especially herbal medicine, seems necessary (7).

One of the richest branches of CAM is Persian medicine (PM). Iranian scholars have paid particular attention to the treatment of menstrual cessation. These treatments include modifications in the lifestyle, herbal medicines, and interventions such as cuppings (8). Amongst herbal remedies proposed for the treatment of oligomenorrhea in the PM textbooks, *Apium graveolens* and *Pimpinella anisum* have attracted increasing attention. The extract of these herbal plants induces menstrual bleeding without leaving noticeable adverse effects (9).

*Apium graveolens* L (Celery) is a member of the Apiaceae family (10). Several studies have proven the analgesic, anti-inflammatory, antioxidative, antihypertensive, antihyperlipidemic, antidiabetic, hepatoprotective, and fertilizer effect of this plant (10-14). Furthermore, *Pimpinella anisum* L. or simply Anise, as a member of the Umbelliferae family, grows in the Eastern Mediterranean region, West Asia, and Iran. Antimicrobial, analgesic, antioxidative, antidiabetic, antihyperlipidemic, and anticonvulsive and neuroprotective effects of this plant, as well as its effect on menopausal hot flashes, have been well studied in various studies (15-18).

Although the positive effects of Celery and Anise hydroalcoholic extracts on hormonal regulation, polycystic ovarian tissue, and fertility have been shown in a few animal studies (19-21), the influence of these medicinal herbs on menstruation has not been scientifically assessed up to now. Thus, this is the first study in this specific medical condition.

## 2. Objectives

Due to the effects of menstrual irregularities on different aspects of women's health, the side effects of chemical and hormonal medications, and the paucity of data on the effects of Celery and Anise combination (CAC) on menstrual disorders in PCOS, we conducted the present study to compare the effects of CAC with those of met. on the amelioration of oligomenorrhea in PCOS patients.

## 3. Methods

### 3.1. Design and Setting

This study was a two-arm randomized triple-blind clinical trial with parallel design performed between February 2015 and February 2016 at the Traditional Medicine Clinic of Sina Hospital (a general-referral government hospital), Tabriz, Iran.

### 3.2. Population, Inclusion, and Exclusion Criteria

The enrolled population included women aged between 18 and 40 years suffering from PCOS with a chief complaint of oligomenorrhea (defined as mense cycles lasting for more than 36 days). The inclusion criteria were as follows: BMI of 18 - 35 kg/m<sup>2</sup>, PCOS according to Rotterdam's criteria, the ability to write and read, willingness to participate in the study, and referral to Sina Hospital. The following items were used as the study exclusion criteria: every pregnant or lactating woman, every patient with any comorbid systemic illness such as cardiovascular, renal, or hepatic diseases, diabetes mellitus, Cushing's syndrome, congenital adrenal hyperplasia, hyperprolactinemia, hypo/hyperthyroidism which commonly accompanies hyperinsulinism and hyperandrogenism, hormone replacement therapy during the last month before the study, malignancies, e.g., ovarian and endometrial carcinomas, and known allergic reactions to the used medications. In addition, if any adverse side effect happened to the enrolled subjects or they needed other treatment modalities such as surgery, they would be excluded from the study.

### 3.3. Study Outcomes

The primary outcome of this study was the regular occurrence of menstrual bleeding during the four cycles of treatment in the study groups. The secondary outcomes were considered to be the treatment complications, the time until the first menstrual bleeding, menstrual cycle regulation score, changes in the menstrual bleeding days and volume, testosterone serum level, luteinizing hormone/follicular stimulating hormone (LH/FSH) ratio, fasting blood sugar (FBS), alanine transaminase (ALT), and aspartate transaminase (AST) serum level after 3 months of treatment. Daily drug intake, detail of menstrual bleeding, and complications were daily assessed by a recording form, a checklist for menstrual changes, and a complication questionnaire. After 12 hours of fasting, a 5 ml blood sample was obtained from the patients to evaluate pregnancy, hematologic, biochemical, and hormonal status before the intervention in 3-5 menstruation days. The sample was then centrifuged at 3000 rpm at room temperature for

5 minutes. Subsequently, LH, total testosterone levels, and LH/FSH ratio were measured using electrochemiluminescence immunoassay (ECLIA). The regulation of menstrual cycles was evaluated with the regularity score points that were previously used (22). Pictorial blood loss assessment chart was used to evaluate bleeding volume.

### 3.4. Study Method

To reach our goal, we primarily selected 146 patients to be included in the study. However, after assessing for the inclusion and exclusion criteria and determining the eligibility of the patients, 72 patients were enrolled in the study using convenience sampling. Subsequently, fixed size block randomization was applied to allocate the patients into one of either met. or CAC group (36 subjects each). Random sequence was determined by Randlist 11 software package. The drugs were prepared in the form of capsules in a uniform shape, size, and color by Shafa Pajhohan Sabz Co. (East Azerbaijan region, Iran). To conceal the allocation, an individual outside of the research team encoded the capsules (A and B), placed them in the closed and opaque packet according to the randomization list, and numbered them consecutively. Placebo capsules comprised lactose powder, and they were used in the CAC group to make the study blind (Double Dummy Technique). Furthermore, capsules containing either met. or CAC or placebo were stored in the same place to reduce CAC-related smell bias. Each subject received an opaque packet containing either capsule of met. in two different colors or capsules of CAC plus placebo in the same color as the met. group. In this study, the researcher, the subjects, and the statistician were blind to the types of medication.

The given demographic data were measured through an interview with the patients, clinical data were obtained by using physical examination and history-taking sheets, and paraclinical variables were assessed through standard laboratory methods.

### 3.5. Study Protocol

Physical and gynecological examinations, uterine and ovarian ultrasonography, and biochemical and hormonal tests such as FSH, LH, thyroid-stimulating hormone (TSH), prolactin (PRL), beta-human chorionic gonadotropin (B-HCG), and testosterone serum level were performed for all patients at the beginning of the study. The diagnosis of PCOS was made by a gynecologist based on the Rotterdam criteria. We also calculated the body mass index (BMI). Laboratory experiments were performed in an academic laboratory. Written informed consent was obtained from all participants. In the first visit, the medication instructions were explained in detail and then, the checklists were

given to the patients to record daily drug intake, complications, and menstrual information.

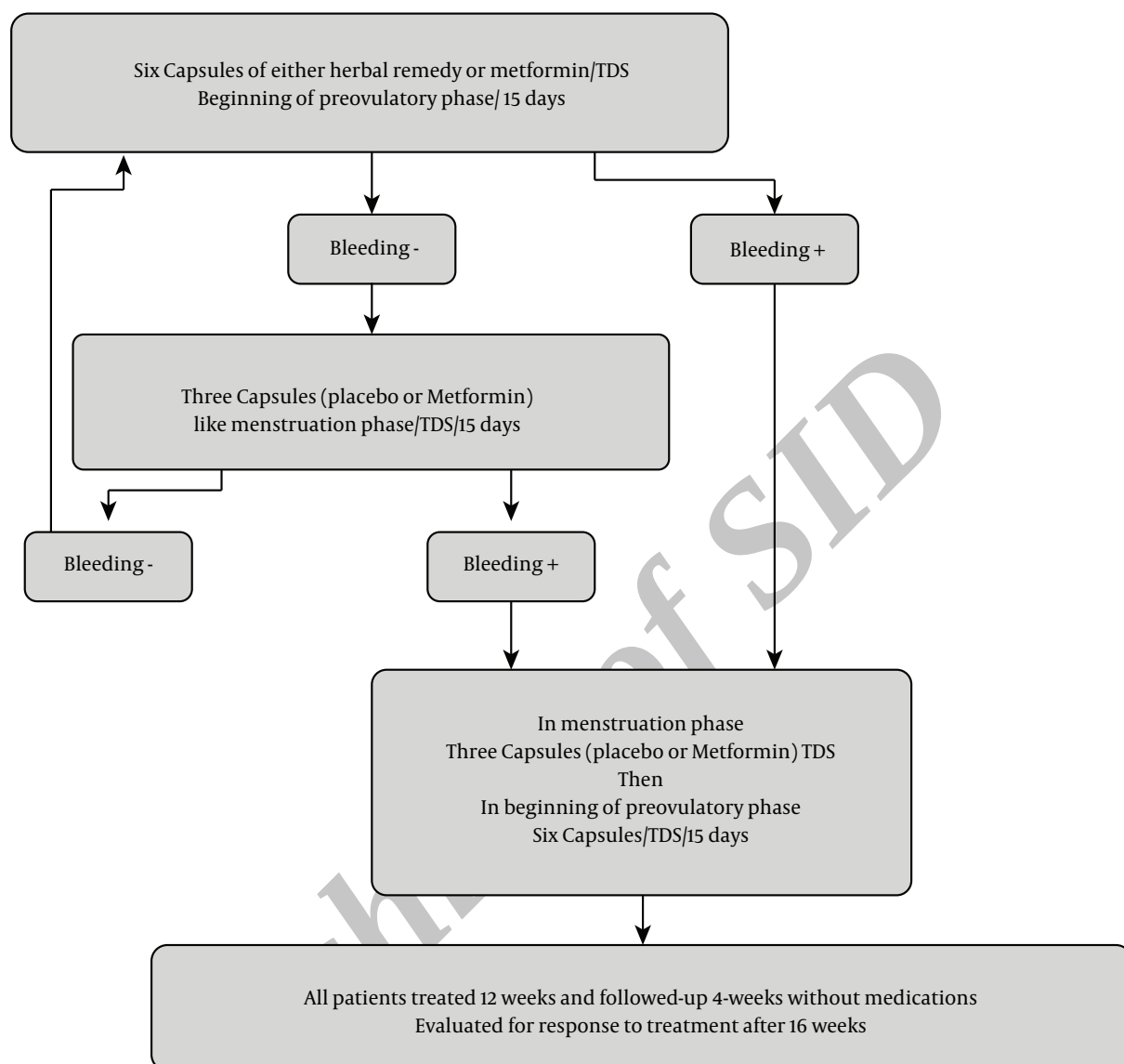
The subjects were administered with six capsules of either CAC or met. (containing 4.5 g/day of seeds powder (23) or 1.5 g/day of met.) at three separate doses for 15 consecutive days beginning after the last day of their spontaneous menstrual bleeding. If the bleeding occurred at any time of using medications, three capsules of either placebo or met. would be administered to the patients during the menstruation phase and then, the follicular phase step with six capsules/day would be repeated. If the bleeding did not occur, as the menstruation phase, three capsules of either placebo or met. would be daily administered to the patients for 15 days until bleeding occurred, and the process mentioned above would be repeated. If the bleeding did not occur up to two weeks, treatment with six capsules/day would start again. The mentioned protocol was repeated for three menstrual cycles and was followed up for four weeks after the treatment (Figure 1). Phone calls were weekly made to track drug intake. In each follow-up visit, regular consumption of medications and probable side effects were evaluated.

### 3.6. Treatment and Plant Samples Processing

Celery and Anise seeds were obtained from a local market and approved by the Herbarium of Faculty of Pharmacy, Tabriz University of Medical Sciences, Iran, and the voucher samples were deposited under herbarium codes, tbz-fph-1744,45. The materials were washed and dried at room temperature and powdered. The microbiological examinations were done to assure their safety for clinical use. Accordingly, we determined the total phenolic compounds (as Gallic acid equivalents) of the plant seeds by Folin Ciocalteu method according to the following equation: Sample absorbance =  $0.0067 \times \text{Gallic acid } (\mu\text{g}) + 0.0132$ , ( $R^2: 0.987$ ) (24). Met. powder was purchased from a local pharmaceutical company and stored at 2 - 5°C temperature.

### 3.7. Statistical Analyses

The principal analysis was done for the primary outcome of the study with the intention to treat (ITT) analysis strategy. We permuted the missing measure through the ITT analysis by moving forward the last observation before the missing case. All the analyses were performed using STATA version 13 statistical software package (StataCorp LP, College Station, TX, USA). Numeric data were expressed as means  $\pm$  standard deviation (SD) and categorical variables were reported as percentages and frequencies. To check the normality of the distribution of numeric measures, quintile-normal plot was used. In case a deviation from the normal distribution was seen to exist, a



**Figure 1.** Diagram depicting the study protocol. TDS, three times a day.

complementary normality assessment test was applied. Independent t-test and Mann-Whitney U test were applied to compare the quantitative data. Chi-square and Fisher's exact tests were used to compare the qualitative data between the two study groups. Paired samples t-test was used to test before-after changes in the values of the numeric data. The relative risk (RR) and the number need to treat (NNT) with their 95% confidence intervals were used for primary outcome assessment. Multiple binary logistic regression was used to control for potential confounding variables in assessing the effect of treatment on the pri-

mary outcome of the study. Survival analysis and Kaplan-Meier curves were used to investigate the effect of treatment on the time to first menstrual bleeding. Cox semi-parametric regression model was used to control for potential confounders. Generalized linear model (GLM) was applied to evaluate the effect of treatment on the trend of bleeding volume and days. The potential confounders considered to be controlled through multivariate analysis included age, body weight, BMI, education, duration of PCOS, testosterone level, and LH/FSH level ratio. Those variables not discovered to have a significant confounding role were

excluded from the final regression model, and the adjusted effect sizes such as odds ratio (OR) or hazard ratio (HR) were reported along with 95% CI. Through all the applied statistical methods, a  $P < 0.05$  was considered statistically significant. All the statistical results were interpreted as two-sided tests of the hypotheses.

### 3.8. Sample Size Calculation

The sample size was estimated using STATA version 14.2 statistical software package. The sample size was calculated based on the Chi-squared comparison of proportions while applying the normal-approximation correction for continuity. The distribution parameters were taken from a blinded comparison of the outcome between the two groups at the end of the first pre-assumed block of 36 participants with the proportions of primary outcome equal to 11% and 51.1% for the comparison groups. A clinical significance margin of 10%, a confidence level of 95%, and a statistical power of 90% were applied to the calculations. Based on the given five parameters, a total sample size of 62 patients was estimated. However, to provide more precision, a final sample size of 72 patients in the two blocks of 36 patients with equal allocation weights for both treatments was decided.

### 3.9. Ethical Approval

The present study complied with all the relevant national regulations, institutional policies, and the requirements of the Declaration of Helsinki and approved by the Ethics Committee of Tabriz University of Medical Sciences, Iran (reference number: TBZMED.REC.1394.530). This clinical trial was registered at the Iranian Registry of Clinical Trials (IRCT2015092413566N6).

## 4. Results

### 4.1. Plant Standardization

Based on the Folin Ciocalteu method, we found that the total phenols of Celery and Anise seeds extracts were respectively 20.86 and 22.86 mg Gallic acid equivalent per 100 g dry plant materials.

### 4.2. General Study Characteristics

In total, 72 patients participated in this clinical trial, and they were randomly allocated to one of the two met. or CAC groups ( $n = 36$  each). However, three patients in each group could not accomplish the study and left the trial due to GI problems, spotting, and so on. ( $n = 33$  each) (Figure 2).

The mean  $\pm$  SD age of the patients was  $26.5 \pm 6.1$  years, and 51.4% of them had an academic education. 62.5% of the patients were married. Most of the participants (91.7%)

were from urban areas. In addition, the mean  $\pm$  SD BMI was  $26.4 \pm 3.5$ . The median (IQR) menarche age was 13 (+2). Menstrual cycle's duration was between 60 and 80 days in 33.3% of the patients. The mean  $\pm$  SD duration of PCOS was  $9.3 \pm 6.7$ . Primary infertility was present in 43.5% of the cases.

There was no statistically significant difference in the baseline demographic, anthropometric, menstrual characteristics, clinical and paraclinical features of PCOS between these two groups except in the education level, LH/FSH ratio, total testosterone level, and duration of PCOS where the difference reached a statistically significant point (Table 1).

### 4.3. Primary Outcome: Menstrual Cycle's Regularity

Our findings showed that menstrual bleeding occurred continuously in 21 patients (58.3%) in the CAC remedy group and nine patients (25%) in the met. group during four cycles of the study. Furthermore, CAC meaningfully increased menstrual cycle's regularity and improved oligomenorrhea compared to the met. group in the study population ( $P < 0.01$ ; NNT, 3; RR, 2.3 with 95% (CI): 1.2 - 4.4). The OR for the improvement of oligomenorrhea in the CAC and met. groups was 3.2 (95% (CI): 1.1 - 4.4). Multiple logistic regression of the confounding variables (education, duration of illness, hirsutism, testosterone level, and LH/FSH level ratio) confirmed that the improving impacts of the CAC are independent of these variables. Further analysis showed that CAC was more effective in patients aged 20 - 25 and a BMI of more than 25.

### 4.4. Secondary Outcomes

Using survival analysis, we found that menstrual bleeding occurred earlier in the herbal remedy group compared to the met. group (estimated median; 24 days vs. 26 days). However, based on the Cox regression model analysis, this finding did not reach a statistical significance (HR=1.4, 95% (CI): -0.82 - 2.2) (Figure 3). Wilcoxon test confirmed that these results were also independent of the confounding variables.

Our results revealed that the regularity score of menstruation was significantly higher in the herbal group than in the met. group ( $P = 0.01$ ) (Table 2).

We also found that more patients in the CAC group than in the met. group had menstrual bleeding after the first treatment periods (81% compared to 68%). This, however, was not statistically significant (Figure 4). Two patients (5.5%) in the herbal remedy group and four patients (11.1%) in the met. group had no menstrual bleeding after three months of treatment.

Longitudinal data analysis using GLM showed that the difference between the bleeding volume of the two study

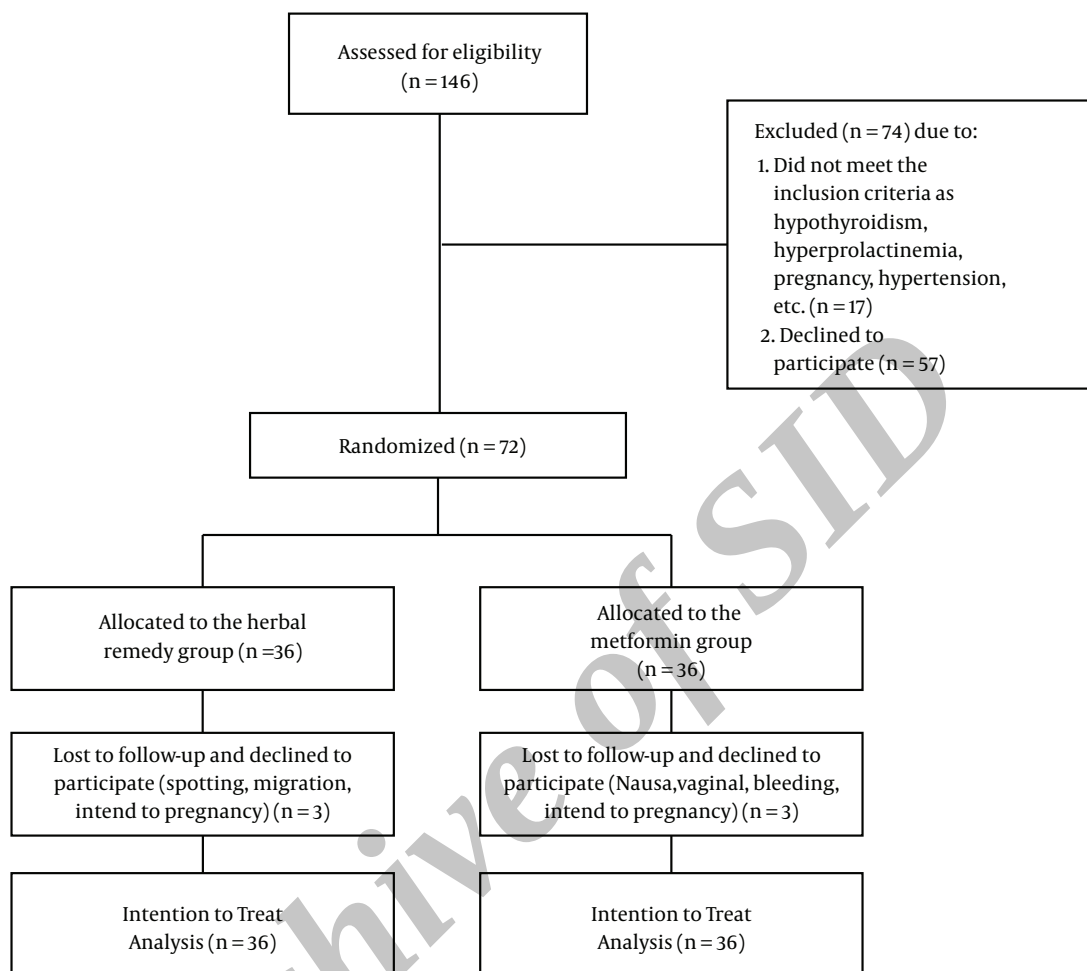


Figure 2. CONSORT statement flow diagram

groups was not significant. However, the same analysis revealed a significant difference in the bleeding days between the two groups ( $P = 0.003$ ).

#### 4.5. LH and LH/FSH Ratio in the Serum

CAC treatment for three months significantly reduced LH serum level (13.4 vs. 9.8;  $P = 0.008$ ). However, there was no remarkable difference in the LH levels between the two study groups at the end of the study.

Treatment with CAC also reduced LH/FSH ratio in the study population (2.8 vs. 2.0;  $P = 0.001$ ). This, however, was not significant in the met. group (2.1 vs. 2.2;  $P = 0.59$ ). Intergroup comparison also showed a statistically remarkable difference in LH/FSH ratio between the two study groups ( $P = 0.002$ ) (Table 3).

#### 4.6. Serum Testosterone Level

CAC administration also decreased the mean testosterone level after the intervention (0.5 vs. 0.3;  $P = 0.001$ ). However, treatment with met. could not reduce the testosterone level in the patients (0.3 vs. 0.4;  $P = 0.51$ ). Intergroup comparison also showed a statistically remarkable difference in the mean testosterone level between the two study groups ( $P = 0.005$ ) (Table 3).

#### 4.7. Sonographic Findings

All of the included patients had the common findings (Rotherham's criteria) of PCOS in their sonographic evaluation of ovaries at the beginning of the study. The results showed that treatment with CAC and met. improved these

**Table 1.** Demographic and Clinical Data of the Included Population<sup>a</sup>

| Variables                   | Anise Plus Celery | Metformin     | P Value |
|-----------------------------|-------------------|---------------|---------|
| <b>Demographic Data</b>     |                   |               |         |
| Age, y                      | 25.5 ± 5.6        | 27.5 ± 6.6    | 0.16    |
| <b>Marital status</b>       |                   |               | 0.22    |
| Single                      | 44.4              | 30.6          |         |
| Married                     | 55.56             | 69.4          |         |
| <b>Education level</b>      |                   |               | 0.00    |
| Academic                    | 69.44             | 33.33         |         |
| Non-academic                | 30.56             | 66.67         |         |
| <b>Habitation</b>           |                   |               | 0.19    |
| Urban                       | 97.22             | 86.11         |         |
| Rural                       | 2.78              | 13.89         |         |
| <b>Job</b>                  |                   |               | 0.34    |
| Housewife                   | 53                | 64            |         |
| Working outside home        | 47                | 36            |         |
| <b>Clinical Data</b>        |                   |               |         |
| <b>Menstrual pattern</b>    |                   |               |         |
| Menarche age, y             | 13.0 ± 2.4        | 13.4 ± 1.5    | 0.42    |
| Menstrual cycle duration, d | 61.30 ± 13.56     | 59.44 ± 12.97 | 0.55    |
| Number of mense days        | 5.3 ± 1.9         | 5.7 ± 1.4     | 0.32    |
| Mense volume, ml            | 43.7 ± 29.1       | 43.6 ± 27.6   | 0.98    |
| <b>PCOS signs</b>           |                   |               |         |
| Hirsutism <sup>b</sup>      | 83.33             | 63.89         | 0.06    |
| Infertility                 |                   |               |         |
| Primary                     | 42.8              | 44            | 0.93    |
| Secondary                   | 9.52              | 12            | 0.79    |
| Duration of infertility, y  | 3.9 ± 3.8         | 5.5 ± 5.8     | 0.45    |
| Duration of PCOS, y         | 7.6 ± 5.5         | 10.9 ± 7.3    | 0.03    |
| Acne, yes/no                | 55.56             | 50.00         | 0.63    |
| Hair loss, yes/no           | 58.33             | 61.11         | 0.81    |
| Family history of PCOS      | 44                | 52            | 0.63    |
| Body weight, kg             | 69.0 ± 11.5       | 69.8 ± 10.6   | 0.77    |
| WHR                         | 0.83              | 0.84          | 0.93    |
| BMI, kg/m <sup>2</sup>      | 26.37 ± 3.9       | 26.52 ± 3.2   | 0.67    |

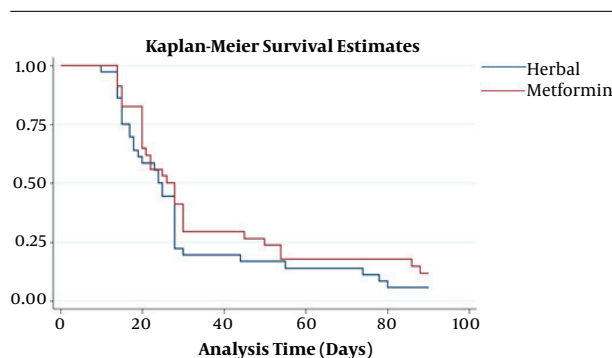
Abbreviations: BMI, Body mass index; PCOS, Polycystic ovary syndrome; WHR, Waist to hip ratio.

<sup>a</sup>Values are expressed as mean ± SD or percentage.<sup>b</sup>Modified Ferriman-Gallwey (mFG) score of ≥ 8 was considered as hirsutism.

findings by 39.4% and 18.2%, respectively. Intergroup comparison revealed no superior effect for CAC over met. ( $P = 0.051$ ).

#### 4.8. Treatments Complications

In this study, no significant side effects were observed in any groups. Although the proportion of mild side effects was the same for both groups (9%), the types of these side effects were different. Constipation ( $n = 1$ ), upset stomach

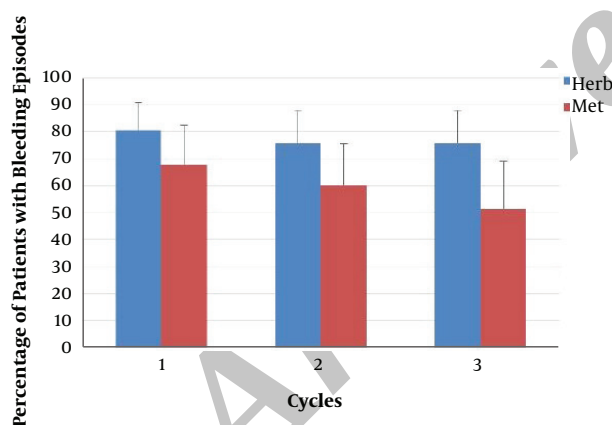


**Figure 3.** Mean duration of treatment with Celery and Anise combination or metformin until menstrual bleeding as demonstrated by Kaplan-Meier graph

**Table 2.** The Regularity Score of Menstruation<sup>a</sup>

| Group     | Mean $\pm$ SD  | 95% Confidence Interval | P Value |
|-----------|----------------|-------------------------|---------|
| Metformin | 2.2 $\pm$ 1.34 | 1.74 - 2.68             | 0.01    |
| Herbal    | 3 $\pm$ 1.2    | 2.59 - 3.41             |         |

<sup>a</sup>Zero means no bleeding episode occurred, one if only one bleeding episode occurred, two if two bleeding episodes occurred, and three if bleeding occurred in all three cycles.



**Figure 4.** Percentage of patients with bleeding episodes per cycle during treatment with herb or met.

( $n=1$ ), and spotting ( $n=1$ ) were reported in the CAC group. Treatment with met. had also side effects such as abdominal pain ( $n=1$ ), nausea ( $n=1$ ), and vaginal bleeding ( $n=1$ ). Besides, the administration of CAC improved dysmenorrhea, bloating, and vaginal infection in some of the patients. Although the number of pregnancies was not surveyed in our study, we found that four and one patients from the CAC and met. Groups, respectively, became pregnant after three months of treatment administration.

## 5. Discussion

To the best of our knowledge, this is the first clinical trial investigating the effect of CAC on menstrual irregularities in PCOS patients and comparing its effects with those of met.

PCOS is a multifactorial disorder. However, evidence suggests that oxidative stress resulted from reactive oxygen species (ROS) production in the follicle can destroy oocytes and cause anovulation, as well as menstrual irregularities (25). Increased ROS generation in PCOS patients may also have a role in its complications that commonly accompany the syndrome (26). Hence, anti-oxidative stress remedies recover normal ovarian function, which leads to the normal ovulation and improvement of menstrual irregularities (25).

In this study, we showed that the administration of met. for three months could improve oligomenorrhea in up to one-fourth of PCOS patients. Similarly, other studies found that the chronic met. administration could improve menstrual irregularities in women with PCOS (27, 28) due to met. anti-oxidant and anti-insulin resistance effects (29). However, met. has side effects such as nausea, diarrhea, and bloating and its use is limited in patients with kidney, liver, and heart diseases (27).

On the other hand, our data revealed that CAC was superior to met. in the improvement of oligomenorrhea and the survival rate of treatment among PCOS patients. Apigenin and apigenin as the main flavonoids of celery seeds exert antioxidative and anti-inflammatory effects. Evidence suggests a direct link between total flavonoid contents of Celery and its antioxidative properties (30, 31). In addition, Aniseed contains 1.5 - 6.0 mass percent of a volatile oil consisting of trans-anethole (75% - 90%) and is considered as an estrogenic agent (32). The essential oil of anise seeds has significant antioxidative and radical scavenging features (33). Phenolic compounds such as flavonoids and flavonols are thought to mediate these properties of anise seeds (34). In addition, these ingredients possess phyto-estrogenic features, which may play a role in this regard and improve menstrual cycles in women with PCOS (35).

Thus, phyto-estrogenic properties and amelioration of oxidative stress can be possible mechanisms by which CAC improved menstrual irregularity in our study.

To our knowledge, no similar studies have yet been performed to assess CAC ingredients for the treatment of oligomenorrhea in PCOS patients (9). Therefore, the results of other studies, which used different herbal medicines for the treatment of oligomenorrhea, were reviewed. The results of the reviewed studies were almost consistent with our findings.

In a clinical trial conducted in India in 2015, a 3-month

**Table 3.** Intergroup Comparison of Secondary Outcomes and Some Other Parameters Before and After Intervention in the Studied Population

| Variables              | Celery + Anise, Before intervention | Celery + Anise, After intervention | P Value | Metformin, Before intervention | Metformin, After intervention | P Value | Celery + Anise, MD (95%CI) | Metformin, MD (95%CI) | P Value |
|------------------------|-------------------------------------|------------------------------------|---------|--------------------------------|-------------------------------|---------|----------------------------|-----------------------|---------|
| LH, mIU/mL             | 13.4                                | 9.82                               | 0.008   | 12.4                           | 10.5                          | 0.56    | 1.86                       | 3.6                   | 0.61    |
| LH/FSH                 | 2.80                                | 2.05                               | 0.001   | 2.16                           | 2.24                          | 0.59    | 0.75                       | -0.08                 | 0.002   |
| Testosterone, ng/mL    | 0.53                                | 0.37                               | 0.005   | 0.38                           | 0.40                          | 0.51    | 0.16                       | -0.02                 | 0.005   |
| BMI, kg/m <sup>2</sup> | 26.37                               | 25.80                              | 0.001   | 26.52                          | 25.75                         | 0.20    | 0.57                       | 0.77                  | 0.57    |
| AST, IU/L              | 18.15                               | 16.54                              | 0.00    | 20.06                          | 18.12                         | 0.00    | -2.80                      | -3.00                 | 0.85    |
| ALT, IU/L              | 17.60                               | 16.21                              | 0.06    | 20.12                          | 17.33                         | 0.02    | -2.77                      | -3.58                 | 0.62    |
| FBS, mg/dL             | 87.40                               | 85.39                              | 0.56    | 85.75                          | 85.43                         | 0.91    | -2.01                      | -0.32                 | 0.86    |

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; FBS, fasting blood sugar; FSH, follicular stimulating hormone; LH, luteinizing hormone; MD, mean difference.

consumption of Fenugreek capsules in 50 women with PCOS aged 18 - 45 years improved menstrual cycles in 71% of the patients and sonographic symptoms in 36 patients and reduced the LH/FSH ratio (36).

In the study of Shahnazi et al., Vitex agnus-castus normalized menstrual cycles in 60% of the patients with PCOS and reduced free testosterone levels after 3 months (37).

The study of Mokaberinejad et al. showed that Mentha longifolia led to menstrual bleeding in 68.3% of the patients with secondary amenorrhea during the first cycle and decreased the LH level (22).

A study revealed that PCOS patients with higher levels of LH/FSH ratio had remarkably higher levels of testosterone but a lower antral follicle count and pregnancy rate. This might be the direct result of LH negative effects on folliculogenesis and receptivity of the endometrium (38).

Our results showed that CAC was more effective than met. in the reduction of the mean testosterone serum level and LH/FSH ratio in PCOS patients. This was in line with the findings of Modaresi et al. study (39), which showed a significant decrease in the testosterone and LH serum levels with Celery use. However, Ghasemiboroon et al. found that Celery could reduce serum LH level in male mice without a significant effect on testosterone and FSH levels (21). Additionally, the results of an animal study showed that anise oil could modify the LH level and improve histological signs of PCOS (19). This may provide another mechanism by which CAC improved menstrual cycles in the studied patients.

In general, our study was a triple-blind clinical trial with a proper method that showed CAC administration, besides improving oligomenorrhea, had mild side effects and in some cases, had some favorable impacts. Thus, it can be used as an emerging novel alternative in the treatment of PCOS. However, the study had a number of limitations. First, we could not perform some para-clinical assays such as estradiol, progesterone, and insulin levels of the patients due to the budget shortage. Second, we could evaluate the mentioned effects in a short period of time, and studies with longer durations are strongly recommended.

## 5.1. Conclusion

In conclusion, our study findings show that orderly administration of Celery and Anise combination efficiently regulates menstrual cycles, and reduces total serum testosterone and LH/FSH ratio in women with polycystic ovary syndrome. We also showed that this effect was superior to that of met. However, further studies with a larger number of cases and more extended follow-up periods are still needed to confirm these results and to elucidate the exact mechanism(s) of action.

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## Footnotes

**Authors' Contribution:** Arezoo Moini Jazani, study conception and design, protocol and project development, data collection, drafting the article, manuscript writing; Hossein Nazemiyeh, study conception, project development, technical and material support, revision of the manuscript; Mojgan Tansaz, study concept and design, protocol and project development; Homayoun Sadeghi Bazargani, study design, data analysis and interpretation of the data, drafting the article, revision of the manuscript; Seyed Mohammad Bagher Fazljou, project development, administrative, technical, and material support; Ramin Nasimi Doost Azgomi, data collection, project development, revision of the manuscript; Kobra Hamdi, study conception and design, protocol and project development, study supervision.

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