Author’s response to reviews

Title: Efficacy and safety of Myrrh in patients with incomplete abortion: a randomized, double-blind, placebo-controlled clinical study

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Responses to the Editor’s and Reviewers’ comments and suggestions

Dear Editor

We are very grateful to the editor for the time and effort has dedicated providing valuable and insightful comments and suggestions on our manuscript. We hope our modifications of the original manuscript have improved the paper to their satisfaction. The changes are made highlighted by yellow in the revised manuscript. In the following, we give a detailed and point-by-point response to the comments.

Editor Comments:

Below are editorial comments:

Comment 1- Please clarify how you tested the normality of your data before doing any statistical tests.
Answer 1- The normality of data has been assessed with the Kolmogorov-Smirnov test (with p value significance level of 0.05).
This sentence has been revised and updated in the statistical analysis section in methods and materials “After assessing the normality of data with Kolmogorov-Smirnov test, independent samples t-test and Chi-square for baseline variables and analysis of variance tests for outcome measurements were used in the statistical analysis” (Page 11, line 238-239)

Comment 2- Numbering should come first in the reference list, not at the end of each reference.
Answer 2- This mistake has been corrected and reference list is now revised.

REVIEWER 1 (Prof Dr. Emaediong Ibong Akpanekpo)

We wish to thank the reviewers for their extensive review and the constructive feedback provided. We hope our modifications of the original manuscript have improved the paper to their satisfaction. Please find below a point by point response to all the issues that were raised and how these were modified in the submitted revision. In the main manuscript, all modifications of the text are emphasized in yellow marker. Also, the manuscript has been edited for proper English language, grammar, punctuation, spelling, and overall style by one of the highly qualified subject-expert native English speaking editors.

Comment 1- Why was 20 weeks used as the gestational age for being included into the study?
Answer 1- Incomplete abortion is a type of abortion where a part of the product of conception is retained within the uterine cavity. The term incomplete abortion is defined as loss of some parts of pregnancy products ≤ 20 weeks of gestation or ≤ 500 gr based on Center for disease control and prevention definition (1). We include patients with gestational age <20 weeks based on LMP calculation and documented first trimester ultrasound scan confirming the gestational age.
“gestational age equal to or smaller than 20 weeks of gestation based on last menstrual period which was confirmed by first trimester ultrasound scan” (Page 8-9, line 175-176)
Comment 2- Is a thickened Endometrial Echo Complex diagnostic or definitive of incomplete abortion?
Answer 2- The main modality for diagnosis of RPOC is color doppler ultrasound, with transvaginal approach being superior to trans-abdominal for accurately detecting RPOC. There are several findings assisting the diagnosis of RPOC by an ultrasound such as thickened endometrial echo, hyperechoic mass suggestive of RPOC, increased vascularity, decreased resistance index, and etc. However, based on the results of the majority of studies, the increased endometrial echo complex (sensitivity 100% and specificity 48%) and/or hyperechoic mass (sensitivity 94% and specificity 77%), are among the best predictors of incomplete abortion and presence of a RPOC (especially when both of these findings are present simultaneously) (2, 3). In general, as written previously in outcome measurement part “A transvaginal color doppler ultrasound has been shown to have a 88% sensitivity and 68% specificity in diagnosis of RPOC” (2) (Page line)

Since these findings in an ultrasound is highly valuable for diagnosis of RPOC, but not definite, this sentence in methods and materials was revised and properly referenced. “The sonographic diagnosis of RPOC was based on the appearance of a thickened endometrial echo complex or the presence of a heterogenous and hyperechoic endometrial mass consistent with RPOC, as has been described in previous studies (26).” (Page 8, line 170-173)

Comment 3- What is the description of the ‘endometrial mass consistent with RPOC’? What features did you use?
Answer 3- A heterogenous and hyperechoic material or mass within endometrial cavity with or without increased vascularity within this echogenic material is consistent with the diagnosis of RPOC in a ultrasound examination (4). “The sonographic diagnosis of RPOC was based on the appearance of a thickened endometrial echo complex or the presence of an heterogenous and hyperechoic endometrial mass consistent with RPOC, as has been described in previous studies (26).”

Comment 4- When you say ‘RPOC with an anterior-posterior diameter 15-50 mm in ultrasound imaging’, what antero-posterior diameter are you measuring? That of the RPOC?
Answer 4- We used a 2D ultrasound with color doppler examination by GE Voluson E6 (GE Medical Systems, Austria) machine with a high-resolution 5-MHz trans-vaginal probe to assess the RPOC anterior-posterior diameter. Method of measurement: After visualizing the RPOC mass in endometrial cavity, the sagittal view of uterus was magnified to cover two-third of the screen. Then the thickness of the RPOC mass, which was the maximum distance across its borders, in the longitudinal plane was recorded. All measurements were taken 2 times with a 5 minutes interval and the mean value was reported as anterior-posterior diameter of RPOC.

Thus, the outcome measurement section was updated and revised as follows: “Ultrasound scans were acquired using a GE Voluson E6 (GE Medical Systems, Austria). After magnifying the image to fill at least two-thirds of the sonogram screen, the maximum distance across the borders of RPOC was measured as an anterior-posterior diameter in a longitudinal plane. Each measurement was taken twice with a 5-min interval and the average values were used in analysis.” (Page 10, line 216-220)
Comment 5- ‘Anterior-posterior diameter was selected between 15-50 mm, because previous clinical trials report favorable outcome after expectant management of RPOC in this range of size’. Anterior-posterior diameter of what? If you used an AP diameter that favors expectant management of RPOC in 84% of cases (from your reference), how were you able to determine that the difference in the outcome of your intended intervention was not due to chance?

Answer 5- The current study was designed as an RCT, controlled by a placebo group in order to eliminate or at least decrease the perceptions and biases in results presentation. The myrrh efficacy in achieving complete abortion after 2 weeks of administration was compared to those receiving placebo in a parallel arm of our study (82.9% vs 54.3%, p=0.01), showing that myrrh’s efficacy was significantly higher than placebo or not attributable to chance. However, as you highlighted, previous studies evaluating the outcome of expectant management for incomplete abortion reported different success rates (from 58% and even up to 84%) (5). This discrepancy is largely due to the different latency periods (from the day of documenting incomplete abortion to the day of achieving complete abortion) used in different studies (from 1 to 10 weeks), the longer the latency period the higher the chance of complete abortion. Therefore, the results of different studies in terms of success rate for complete abortion should be compared with each other very cautiously since there are lots of variations in clinical settings, latency intervals, and inclusion/exclusion criteria. In a meta-analysis, the expectant-care group was significantly more likely to have RPOC or incomplete abortion by two weeks when compared to intervention (D&amp;C) (RR= 3.98) (6).

Nevertheless, the goal of any intervention (myrrh therapy in this unique study) for incomplete abortion cases is to address and resolve this issue in a shorter interval in order to prevent long-term medical (such as pelvic infection, pain, unplanned surgeries) and psychological complications related to incomplete abortion (6).

“In summary, the current evidence does not indicate the superiority of either expectant care (no treatment) or other interventions for incomplete abortions. However, if a woman chose the expectant management, she should be fully informed regarding the higher rate of RPOC, need for unplanned surgical evacuation, blood transfusion, pelvic infection, etc. (9).” (Page 15, line 322-325)

Comment 6- Did you use a computer program to generate the numbers? What is the name of this program?

Answer 6- The study population was randomized into two groups by using computer-generated random numbers (www.randomizer.org, an online randomization tool). (Page 9, line 188)

Comment 7- How was the sample size determined?

Answer 7- Due to the lack of previous study, the strategy of sample size calculation was based on a pilot experiment conducted in our center on 10 patients which showed a mean difference of -6.7±10 mm in AP diameter of RPOC after administration of myrrh (7) [reference of the statistical software used for sample size calculation based on our pilot results of mean reduction of RPOC size]. By considering α=0.05 and power=%80, sample size was calculated to be 35 women in each group, but was increased to 40 individuals considering possible dropouts.
“Due to the lack of previous studies, the strategy of sample size calculation was based on a pilot experiment conducted in our center on 10 participants who showed a mean difference of -6.7±10 mm in AP diameter of RPOC after administration of myrrh. Then, by entering these pilot results in a statistical tool named “Sample Size Calculator for Comparing Two Independent Means” (31), sample size in each group was calculated as 35 patients for each group based on 80% power and an error of 5% (P<0.05, two-sided). To compensate for possible dropouts, we recruited 40 patients in each group.” (Page 11, line 229-235)

Comment 8- How did you determine the dose of Myrrh? Can you provide references?
Answer 8- There is no previous or at least similar trial of myrrh for use as a reference for dose determination. Therefore, we use 2 methods for determining our optimal dose of 500 mg three times a day, 1500 mg per day, (divided as every 8 hours). 1) the ethnomedicine use of myrrh in Iran: as noted in introduction of the current manuscript the myrrh is noted in Traditional Persian Manuscripts as a herbal remedy as abortifacient and assisting the expulsion of retained tissues (RPOC) possibly by causing uterine stimulation, and in TPM manuscripts (in Farsi) (8) the recommended dose is approximately equal to 1500 mg per day. Therefore, local herbal shops and traditional healers follow the same rule and prescribe the same amount to women since old times. 2) we search the literature and record the different doses of myrrh used in previous trials for other purposes and evaluated the side-effects happened after each dose, El-sherbiny et al. (9) used 1200 mg (2 capsules 600 mg) every 24 hr for trichomoniasis vaginalis infection, Langhorst et al. (10) used 1200 mg every 24 hr for ulcerative colitis. Interestingly, no proven unexpected drug reactions occurred during these studies. It seems this range of doses of myrrh can be considered safe for clinical trials.

Comment 9- What were the expected side effects and how was it managed or prevented?
Answer 9- Gastrointestinal, hypersensitivity (allergic) reactions and malaise/ tiredness were among the reported side effects of myrrh use by human subjects (11); however, a greater number of clinical trials report no noticeable side effect related to myrrh therapy (12, 13). After an extensive search through the literature, we designed a questionnaire consisting from open-ended questions covering GI symptoms (nausea, vomiting, diarrhea, constipation, epigastric pain, fullness, etc.), Allergic reactions (i.e., skin and respiratory manifestations), palpitation, and weakness and malaise. Therefore, acknowledging these potential side effects, at the baseline visit of the trial, patients were fully informed regarding the potential sign and symptoms of these adverse effects. Patients were contacted by telephone every 2 days and visited by a physician on a weekly basis until the end of study and also the were provided with a 24/7 telephone number of a resident physician for any emergency conditions requiring medical assistance. The patients from both groups who did not achieve complete abortion at the end of study were offered routine protocols of incomplete abortion according to guidelines of our teaching hospital.

Besides to informing participants about myrrh side effects, all patients were informed about the complications of incomplete abortion such as increasing vaginal bleeding, malodor vaginal discharge, and other signs and symptoms of infection (endometritis) and then instructed to visit the nearest healthcare center for admission and receiving proper treatment. However, during the study period no endometritis happened.

“After explaining myrrh-related side effects as well as signs and symptoms of endometritis (as a complication of incomplete abortion), patients were followed with alternating telephone calls every 2 days to assure treatment compliance and screening for any adverse events with asking open-ended questions.” (Page 10, line 207-209)
Comment 10- What adverse events did you screen for?
Answer 10- As mentioned in previous answer, the expected side effects which were discussed with patients at the beginning of trial, were repeated at each phone call and in-person visit (asked by physician). Open-ended questions were asked in terms of gastrointestinal symptoms, vaginal bleeding (daily pad count), palpitation, cramps/abdominal pain, signs and symptoms related to endometritis such as malodor pussy discharge, chills and fever.

Comment 11- Did you measure the AP diameter of the endometrial cavity at the site of the suspected RPOC or did you measure the diameter of the RPOC? Can you provide references to show that this measurement is an adequate measure or predictor of complete uterine evacuation?
Answer 11 – Measuring the anterior-posterior (AP) diameter of endometrial cavity at the site of RPOC would result in a falsely over-estimated measurement of RPOC size, since endometrial thickness may be included in our measurement as well. Therefore, the uterus in axial view was magnified enough (cover 2/3 of screen, as discussed earlier) to visualize and differentiate the endometrial line from RPOC-mass border. Then, we try to put calipers on the outer border of AP diameter of RPOC mass to minimize the chance of over- or under-estimation of size. This measurement was previously discussed in our manuscript in page 10 line 216-220.

Answer Part 2- In a cohort study in year 2005, the size of RPOC were measured serially until resolution of the pregnancy (in order to show the predictive value of RPOC size in spontaneous resolution). The results of the study showed that women with smaller RPOC sizes were more susceptible to have a complete resolution without any complication as compared to those with larger RPOC who would end up being evacuated by dilation and curettage (18.6 mm vs. 24.7 mm, p<0.05) (14).
“Furthermore, the decreased measures of AP-diameter of RPOC has been shown to be an indicator of complete spontaneous resolution (30).” (Page 10 line 222-223)

Comment 12- Were the residuals normally distributed prior to using the independent t test? What tests of normality did you use to ensure this?
Answer 12- Yes data were checked for normality of distribution and was normally distributed.
The normality of data has been assessed with the Kolmogorov-Smirnov test (with p value significance level of 0.05).
This sentence has been revised and updated in the statistical analysis section in methods and materials “After assessing the normality of data with Kolmogorov-Smirnov test, independent samples t-test and Chi-square for baseline variables and analysis of variance tests for outcome measurements were used in the statistical analysis” (Page 11, line 238)

Comment 13- Did you test for homogeneity of variance? What test did you use?
Answer 13- Yes. Prior to making the relevant calculations, the assumption of homogeneity of variance was checked via Levene's test.

Comment 14- How did you evaluate the safety profile of Myrrh in this study?
Answer 14- safety profile assessment during the 2 weeks of our study was evaluated using a previous study entitled “Drug safety assessment in clinical trials: methodological challenges and opportunities”(15) (specifically safety of drugs for randomized clinical trial).
Briefly, in this clinical trial we explain and then monitor for prespecified adverse events based on pharmacological mechanisms or data from earlier studies of myrrh (as reported in previous answers such as gastric and allergic side-effects) and these prespecified adverse
outcomes and safety concerns were gathered as a safety questionnaire and then checked with patients at each phone call/in-person visit. However, considering new or unexpected adverse effects an open-ended question was designed at the end of questionnaire asking “is there any other complaint or discomfort emerged after start of using myrrh which was not asked you in previous questions?”

Comment 15- ‘Findings of the current study showed that the complete abortion rate was 82.9% for the intervention group.’ What was your outcome measure? What value of your outcome measure is suggestive of a ‘complete abortion’?

Answer 15- In clinical trials assessing managements for incomplete abortion the term “complete abortion or full expulsion” or other equivalents (as the final goal / aim of the study), is a condition sonographically defined as no sign of RPOC mass in ultrasound exam at end of study and only homogenous endometrial lines being seen in ultrasound examination. In other terms, A complete miscarriage is “an empty uterine cavity in women with conclusive evidence of RPOC on her previous scans”(14)

“As a secondary outcome measure, the rate of complete abortion was identified for the both groups at the end of study, which is defined as an empty uterine cavity in with no visual sign RPOC in the ultrasound imaging of a woman with conclusive evidence of RPOC in her previous scans (30).” (Page 11, line 224-227)

REVIEWER 2 (Prof Dr. Yoon Jae Lee, PhD)

We wish to thank the reviewers for their extensive review and the constructive feedback provided. Please find below a point by point response to all the issues that were raised and how these were modified in the submitted revision. In the main manuscript, all modifications of the text are emphasized in yellow marker.

Comment 1- I would like to know the Myrrh had extracted or just had powdered. (Please add the detailed information in method). If the Myrrh had just powdered, this type of administration is unlikely to guarantee that the subjects received the same dose of Myrrh.

Answer 1- Since there was no previous study examining which part of myrrh is effective in treatment of incomplete abortion (oil or gum or resin), therefore extracting was not done in our study. Based on recommendations from ethnomedicine of Iran, after removing leaves and other impurities, the cleaned oleo-gum-resin of myrrh was grounded to a powder then sieved through a mesh size of 40 to ensure its uniformity and each capsule contained 500±25 mg. since our clinical trial confirm the efficacy of myrrh oleo-gum-resin as a whole, and not determined which active compound is responsible for its efficacy. Therefore, further well designed in vitro or animal studies on extracts of different parts of oleo-gum-resin of myrrh are recommended to show their effects on uterine smooth muscle’s contractility and other potential mechanisms related to its application in causing abortion and RPOC (retained products of conception) expulsion.

Comment 2- I think the authors need to change conclusions in abstract more obliquely.

Answer 2- Dear reviewer the conclusion part in abstract now has been changed and we interpreted our study’s results with more caution. “This study shows that Myrrh is effective and safe in resolution of the RPOC and may be considered as an alternative option for treatment of patients with incomplete abortion.
However, further studies on active compounds isolated from myrrh and their uterine stimulant effects are needed.” (Page 4, line 75-78)

Comment 3- Are there any toxicological studies of Myrrh that have been conducted? No serious side effects have occurred in this trial, but I think that the references of toxicological studies would be required in discussion. Answer 3- The main results of a recent toxicological experiment on myrrh in animal model is presented in discussion as follows:
“No serious side effects were reported in previous trials using myrrh on human subjects (39, 40). However, a toxicological evaluation of Myrrh essential oil was conducted in mice and shown that lower doses of myrrh (1, 5, and 10 μL) did not cause skin inflammation, swelling, dermatitis, scabbing, and abrasions. The liver and kidney enzymes were also found in normal range which was comparable to the control group. On the other hand, in acute toxicological analysis, subcutaneous injection of higher doses of myrrh (20, 40, and 80 μL) adverse hepatic, renal and allergic events were noted in animal models (46).” (Page 14-15, line 314-321)


