Nephro-Urology Monthly
Volume 17 · Issue 4 · e165999

OPEN PEER REVIEW

Folic Acid as a Potential Adjunct in the Management of Primary Nocturnal Enuresis: A Clinical Trial Study

Parsa Yousefichaijan, Masoud Rezagholizamenjany, Fatemeh Dorreh, Manijeh Kahbazi, Ali Arjmand Shabestari

SUBMITTED	REVISED	ACCEPTED	DOI
3 Sep 2025	18 Oct 2025	9 Nov 2025	10.5812/numonthly-165999

About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

5 comments · 7 Sep 2025 – 18 Sep 2025

Referee 1 Reviewer

MAJOR REVISION 7 Sep 2025

Dear Authors,
You report a trial of folic-acid supplementation versus control in 68 children with primary nocturnal enuresis and claim clinically meaningful increases in “dry days” at Month-1 and Month-2. You present group means, Cohen’s d effect sizes, and p-values from independent t-tests. The idea is interesting, but the manuscript in its present form has several serious methodological, statistical, and reporting defects that prevent any favorable publication decision without major revision.

Major concerns (each must be addressed in a revision or will result in rejection)

1. Fundamental inconsistencies about trial registration, duration, and design.
The manuscript contains conflicting registration/ethical details (different IRCT codes are listed in different parts of the file) and conflicting descriptions of duration: the Abstract says “four weeks” while the Methods and other places describe 60 days (two months). The Abstract says “randomized … placebo” but the Methods say “allocated in two sequential blocks” and the control group “continued standard care without folic acid supplementation” (no clear placebo or blinding).
These contradictions are major and need one clear, consistent statement of (a) the exact trial registration number and registry entry, (b) dates of enrollment, (c) the planned treatment duration, and (d) whether a placebo was used and whether participants, caregivers, and outcome assessors were blinded. Please correct and harmonize all parts of the manuscript and provide the original registry entry (link to the registry page) that shows the pre-specified primary outcome and analysis plan.
2. Randomization, allocation concealment, and blinding are not described (or descriptions are contradictory).
The Abstract calls the study “randomized,” but the Methods suggest sequential block allocation; there is no description of the random sequence generation method, allocation concealment, block size, who performed randomization, or any blinding.
For a trial claim, these items are essential. If the trial was randomized, the authors must state the exact randomization procedure and how blinding (if any) was maintained. If not randomized, the manuscript must not call itself randomized, and the design should be described correctly.
3. Primary outcome and statistical analysis plan were not prespecified / reported.
The manuscript does not clearly state a pre-specified primary outcome (is it a change in monthly dry days? proportion dry? other?). It appears that multiple comparisons (baseline, Month-1, Month-2) were tested by separate independent t-tests. This multiplicity and the use of independent tests on repeated measures are methodologically inappropriate and inflate Type I error.
Authors must (a) state the primary outcome and the primary time point as pre-specified in registration, (b) provide the statistical analysis plan (pre-defined), and (c) reanalyze with an appropriate model for repeated measures (see next point).
4. Incorrect statistical approach for repeated measures and missing confounder adjustment.
4.1. Using independent-samples t-tests at each time point is suboptimal because measurements are repeated within subjects and the within-subject correlation is ignored. The correct inferential approach is a model that accounts for within-subject correlation (for example: repeated-measures ANOVA with Group×Time interaction or, preferably, a linear mixed-effects model with random intercepts for subjects). The model should test the group × time interaction (this is the inferential test of treatment effect over time).
4.2. Authors should provide individual-level data and reanalyze using a mixed model (or ANCOVA adjusting

for baseline), present adjusted mean differences with 95% confidence intervals, and report effect sizes with CIs. Also, do sensitivity analyses (per-protocol and ITT with appropriate methods for missing data, e.g., multiple imputation). Provide raw numbers of missing values at each time point and how they were handled.
5. Absence of individual-level data for verification.
The text states "individual-level data were not available," which is not acceptable for a trial manuscript: the authors must supply an anonymized individual-level dataset to the editors (and preferably as online supplementary material) so that results can be verified and reanalyzed. If there is a justifiable reason for not releasing data publicly, at a minimum, the dataset must be available to the editorial office and reviewers.
6. Sample size calculation is inadequately reported.
The manuscript states a sample size of 34 per group was calculated (citing Hajalmass), but gives no formula, no assumptions (expected difference, SD, alpha, power), and no allowance for attrition. The authors must present the full sample-size calculation, including the clinical effect size they aimed to detect and the source of the SD estimate.
7. Outcome definition, units, and clinical relevance need clarity.
It is not explicitly stated what "dry days" means (number of dry nights in a 30-day window? calendar days?). Tables suggest means around 19 at baseline—please define the denominator and measurement window, how data were recorded (diary by parents?), and whether the outcome was validated. Also, authors should report clinically meaningful responder definitions (e.g., ≥50% reduction, complete dryness) and present responder rates and absolute risk differences/NNT with 95% CI.
8. No baseline folate (or B12) measurement and potential confounding.
The biological rationale hinges on folate deficiency; yet, no baseline serum folate (or B12) or dietary assessment is reported. Without baseline nutrient status, it is difficult to interpret whether improvement is due to correcting the deficiency or a nonspecific placebo/context effect. Authors should (if possible) provide baseline folate/B12 measurements or explain why they were not measured; at a minimum, discuss this limitation more strongly. Also consider adjustment for important clinical confounders (age, sex, BMI, presence of sleep apnea/constipation, developmental delay).
9. Safety, compliance, and adverse events are insufficiently reported.
For pediatric supplementation, authors must report compliance (how many doses were taken, pill counts, or parental report), any adverse events, and monitoring procedures. The manuscript currently lacks these details.
10. Reporting standards and transparency (CONSORT) are not met.
A clinical trial must follow CONSORT: include a CONSORT flow diagram with numbers screened, excluded (with reasons), randomized, lost to follow-up, and analyzed (ITT vs PP). Include the CONSORT checklist as a supplement. Provide exact p-values to three decimals (do not present "0.0000"; use "$p < 0.001$") and give 95% confidence intervals for estimated effects. Report effect sizes with 95% CI.

Summary Note
The manuscript addresses an interesting and potentially useful clinical question. Nevertheless, the current manuscript contains several severe methodological and reporting flaws. I recommend a major revision with full compliance with CONSORT, supply of raw data, and a reanalysis as specified above.
Kind regards,

Referee 2 Reviewer

MINOR REVISION 9 Sep 2025

1- The references are general and old (e.g., 1, 2, 3…) but it is not clear whether their sources are up-to-date (2020 onwards). It would be better to add at least a few new references.
2- Sampling method: The use of convenience sampling can introduce selection bias. It would have been better

to explain randomization and allocation concealment. The text says "sequential blocks" (line 70), but the exact randomization method (block randomization? stratified?) is not clear. This is a major weakness.

3- Blinding: There is no mention of blinding (researcher or participant). Even if the study was open-label, it should have been mentioned.

4- Outcome measure: Only "weekly frequency of enuresis episodes" is mentioned. It would have been better to use a standardized or validated tool (e.g., a bladder diary or quality of life questionnaires).

5- Duration of follow-up: Only one month after the start of treatment was assessed (lines 76–77), while the intervention was 60 days. This makes the long-term effect of the intervention unclear.

Statistical details:

6- The correct statistical tests were chosen, but the method for checking the normality of the data (e.g., Shapiro–Wilk) was not mentioned.

7- Management of missing data or dropouts was not reported.

8- Dose chosen: A dose of 1 mg of folic acid per day was chosen, but no explanation was given for the basis of choosing this dose (clinical guidelines or previous studies).
Dropout / Adherence reporting:

9- It is not specified how many people dropped out of the study (attrition) or what the compliance with the intervention (compliance with folic acid) was. This could introduce bias.

Focus only on Dry Days:

10- Secondary outcomes were not reported (e.g., quality of life of the child or family, severity of episodes, side effects of the drug). This limits the robustness of the results.

Incomplete statistical details:

11- The exact test used for the repeated measures data (month 1 and month 2) is not clear. Only the independent t-test is mentioned in the Methods, but here repeated measures ANOVA or mixed models would have been more appropriate.

12- The confidence interval (CI) for the difference in means is not reported; this is essential in reputable articles.

13- No mention is made of the lack of blinding and randomization limitations. This was one of the most important weaknesses of the Methods and should also be mentioned in the Discussion.

Structure of the text:

14- Sections are long and could be divided into shorter paragraphs for better readability.

15- In some places (lines 151–156), the literature is vague and repetitive.

Referee 3 Reviewer

MINOR REVISION 11 Sep 2025

Dear AE,
Thank you for the invitation.
The manuscript addresses an interesting and potentially clinically relevant topic: the role of folic acid supplementation in primary nocturnal enuresis (PNE). The rationale linking neurodevelopment, folate status, and bladder control is well described, and the study attempts a randomized controlled trial (RCT) design, which is appropriate for testing an intervention. Here I have some suggestions and comments that

should be addressed:
1-The manuscript mentions sequential block allocation but does not clarify whether allocation was concealed or whether participants/assessors were blinded.
2-“Dry days” as a metric is meaningful but lacks a standardized operational definition across studies.
3-In the Discussion, neurodevelopmental mechanisms linking folate to PNE are speculative; the study does not measure folate levels or neurophysiological markers.
4-References to neuroimaging and CNS maturation are not directly supported by data from the current study.
5-In all tables, the abbreviated words must be explained in a legend.
6-Grammatical inconsistencies exist in a few places (e.g., “over a four weeks period” → “over a four-week period”).

Associate Editor

MAJOR REVISION 11 Sep 2025

Dear Editor-in-Chief,

I would like to thank the authors and express appreciation for their contribution. The manuscript represents valuable research; however, some revisions are necessary. The authors should address the reviewers’ comments and make the required adjustments. Additionally, minor corrections are needed in the abstract:

*Minor grammatical issues (e.g., “over a four weeks period” → “over a four-week period”; “occurance” → “occurrence”).
*The abstract does not mention whether the trial was blinded or whether allocation was concealed, which is critical for RCT quality assessment.
*“Urinary control parameters” are vaguely defined. Specific parameters should be briefly mentioned.

Editor-in-Chief

MAJOR REVISION 18 Sep 2025

Major Revision

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

With Greetings

REVIEWER No. 0:

Comment 1:

Fundamental inconsistencies about trial registration, duration, and design.

The manuscript contains conflicting registration/ethical details (different IRCT codes are listed in different parts of the file) and conflicting descriptions of duration: Abstract says “four weeks” while Methods and other places describe 60 days (two months). The Abstract says “randomized … placebo” but Methods say “allocated in two sequential blocks” and the control “continued standard care without folic acid supplementation” (no clear placebo or blinding).

These contradictions are major and need one clear, consistent statement of (a) the exact trial registration number and registry entry, (b) dates of enrollment, (c) the planned treatment duration, and (d) whether a placebo was used and whether participants, caregivers, and outcome assessors were blinded. Please correct and harmonize all parts of the manuscript and provide the original registry entry (link to the registry page) that shows the pre-specified primary outcome and analysis plan.

Answer:

Mentioned errors were corrected and highlighted. IRCT20130518013366N14, we have placebo control and treatment period was 60 days, and evaluation was done in this period.

Comment 2:

Randomization, allocation concealment, and blinding are not described (or contradictory).

The Abstract calls the study “randomized,” but the Methods suggests sequential block allocation; there is no description of the random sequence generation method, allocation concealment, block size, who performed randomization, or any blinding.

For a trial claim, these items are essential. If the trial was randomized, authors must state the exact randomization procedure and how blinding (if any) was maintained. If not randomized, the manuscript must not call itself randomized, and the design should be described correctly.

Answer:

We clarify randomization and blinding methods.

Comment 3:

Primary outcome and statistical analysis plan were not prespecified / reported.

The manuscript does not clearly state a pre-specified primary outcome (is it a change in monthly dry days? proportion dry? other?). It appears multiple comparisons (baseline, Month-1, Month-2) were tested by separate independent t-tests. This multiplicity and the use of independent tests on repeated measures are methodologically inappropriate and inflate Type I error.

Authors must (a) state the primary outcome and the primary time point as pre-specified in registration, (b) provide the statistical analysis plan (pre-defined), and (c) reanalyze with an appropriate model for repeated measures (see next point).

Answer:

We thank the reviewer for highlighting this important point. We have now clearly defined the primary outcome as the mean change in the number of dry nights per 30-day period. The primary time point was Month 2. This has been specified in the Statistical Analysis section (highlighted in yellow). We have also described that the statistical analysis plan was defined a priori and registered in the Iranian Clinical Trial Registry. The revised analysis now uses a linear mixed-effects model with baseline adjustment to account for repeated measures and within-subject correlation.

Comment 4:

Incorrect statistical approach for repeated measures and missing confounder adjustment.

4.1. Using independent-samples t-tests at each time point is suboptimal because measurements are repeated within subjects and the within-subject correlation is ignored. The correct inferential approach is a model that accounts for within-subject correlation (for example: repeated-measures ANOVA with Group×Time interaction or, preferably, a linear mixed-effects model with random intercepts for subjects). The model should test the group × time interaction (this is the inferential test of treatment effect over time).

4.2. Authors should provide individual-level data and reanalyse using a mixed model (or ANCOVA adjusting for baseline), present adjusted mean differences with 95% confidence intervals, and report effect sizes with CIs. Also, do sensitivity analyses (per-protocol and ITT with appropriate methods for missing data, e.g., multiple imputation). Provide raw numbers of missing values at each time point and how they were handled.

Answer:

We agree with the reviewer's concern. The original analyses relied on t-tests for descriptive purposes, but in the revised manuscript we have re-analyzed the data using a linear mixed-effects model with random intercepts and Group × Time interaction. This model accounts for repeated measures and within-subject correlation. Baseline values were included as covariates. We now report adjusted mean differences, 95% confidence intervals, and effect sizes. Sensitivity analyses (per-protocol and ITT with multiple imputation for missing data) have also been added. Details appear in the Statistical Analysis and Results sections.

Comment 5:

Absence of individual-level data for verification.

The text states "individual-level data were not available," which is not acceptable for a trial manuscript: the authors must supply an anonymized individual-level dataset to the editors (and preferably as online supplementary material) so that results can be verified and re-analysed. If there is a justifiable reason for not releasing data publicly, at a minimum, the dataset must be available to the editorial office and reviewers.

Answer:

We acknowledge the importance of transparency. We have now prepared an anonymized individual-level dataset, which will be made available to the editorial office and reviewers. If

permitted, we are also willing to provide this as supplementary material for readers. This is clarified in the revised manuscript.

Comment 6:

Sample size calculation is inadequately reported.

The manuscript states a sample size of 34 per group was calculated (citing Hajalmass), but gives no formula, no assumptions (expected difference, SD, alpha, power), and no allowance for attrition. The authors must present the full sample-size calculation, including the clinical effect size they aimed to detect and the source of the SD estimate.

Answer:

We appreciate this observation. The Methods section has been revised to include the full sample size calculation, including the expected effect size (3 dry nights per 30 days), standard deviation assumption (4), alpha (0.05), power (80%), and an additional 10% allowance for attrition. The revised description is highlighted in yellow.

Comment 7:

Outcome definition, units, and clinical relevance need clarity.

It is not explicitly stated what “dry days” means (number of dry nights in a 30-day window? calendar days?). Tables suggest means around 19 at baseline—please define the denominator and measurement window, how data were recorded (diary by parents?), and whether the outcome was validated. Also, authors should report clinically meaningful responder definitions (e.g., ≥50% reduction, complete dryness) and present responder rates and absolute risk differences/NNT with 95% CI.

Answer:

We agree that the outcome definition required more precision. We have now clarified that “dry days” were defined as the number of nights without enuretic episodes during a 30-day observation period, recorded daily by parents in standardized diaries and validated against clinical interviews. We also added responder definitions (≥50% reduction and complete dryness) and report responder rates with risk differences and NNTs. These revisions are included in the Methods and Results sections (highlighted in yellow).

Comment 8:

No baseline folate (or B12) measurement and potential confounding.

The biological rationale hinges on folate deficiency; yet, no baseline serum folate (or B12) or dietary assessment is reported. Without baseline nutrient status, it is difficult to interpret whether improvement is due to correcting the deficiency or a non-specific placebo/context effect. Authors should (if possible) provide baseline folate/B12 measurements or explain why they were not measured; at a minimum, discuss this limitation more strongly. Also consider adjustment for important clinical confounders (age, sex, BMI, presence of sleep apnea/constipation, developmental delay).

Answer:

We acknowledge this limitation. Unfortunately, baseline serum folate and B12 levels were not measured in our trial due to resource constraints. We now explicitly state this as a limitation in the Discussion and emphasize the need for future studies to include baseline nutrient status and adjust for relevant confounders (age, sex, BMI, comorbidities).

Comment 9:

Safety, compliance, and adverse events are insufficiently reported.

For pediatric supplementation, authors must report compliance (how many doses taken, pill counts, or parental report), any adverse events, and monitoring procedures. The manuscript currently lacks these details.

Answer:

We thank the reviewer for this suggestion. We have now added a dedicated Safety and Compliance section in the Results. Compliance exceeded 95% in both groups (assessed via pill counts and parental diaries). No serious adverse events were reported. Minor gastrointestinal discomfort occurred in 3 children across groups, but all completed the study. This section is highlighted in yellow.

Comment 10:

Reporting standards and transparency (CONSORT) are not met.

A clinical trial must follow CONSORT: include a CONSORT flow diagram with numbers screened, excluded (with reasons), randomized, lost to follow-up, and analysed (ITT vs PP).

Include the CONSORT checklist as a supplement. Provide exact p-values to three decimals (do not present “0.0000”; use “$p < 0.001$”) and give 95% confidence intervals for estimated effects.

Report effect sizes with 95% CI.

Answer:

We agree. We have now revised the manuscript to align with CONSORT reporting standards. Specifically, we:

- Prepared a CONSORT flow diagram (included as supplementary material).
- Added a CONSORT checklist.
- Reported exact p-values (to 3 decimals, with “$p < 0.001$” where appropriate).
- Reported adjusted effect sizes with 95% confidence intervals.

We believe these changes substantially improve the clarity, transparency, and rigor of the manuscript.

REVIEWER No. 1:

Comment 1:

The references are general and old (e.g. 1, 2, 3…) but it is not clear whether their sources are up-to-date (2020 onwards). It would be better to add at least a few new references.

Answer:

The study used the most recent possible references; however, some older sources were revised.

Comment 2:

Sampling method: The use of convenience sampling can introduce selection bias. It would have been better to explain randomization and allocation concealment. The text says "sequential blocks" (line 70), but the exact randomization method (block randomization? stratified?) is not clear. This is a major weakness.

Answer:

We clarified in Methods that participants were enrolled by convenience sampling. Randomization was performed using computer-generated block randomization (block size = 4), with allocation concealment ensured via sealed opaque envelopes. This limitation is acknowledged in Discussion.

Comment 3:

Blinding: There is no mention of blinding (researcher or participant). Even if the study was open-label, it should have been mentioned.

Answer:

We confirmed the trial was double-blind: participants, caregivers, outcome assessors, and data analysts were blinded.

Comment 4:

Outcome measure: Only "weekly frequency of enuresis episodes" is mentioned. It would have been better to use a standardized or validated tool (e.g. bladder diary or quality of life questionnaires).

Answer:

We defined “dry days” as nights without enuresis, recorded in standardized parent diaries validated by clinical interview. We acknowledge the absence of standardized QoL measures as a limitation.

Comment 5:

Duration of follow-up: Only one month after the start of treatment was assessed (lines 76–77), while the intervention was 60 days. This makes the long-term effect of the intervention unclear.

Answer:

Final evaluation was conducted at two months, matching the treatment duration. We acknowledge that longer follow-up is required to confirm sustainability.

Comment 6:
The correct statistical tests were chosen, but the method for checking the normality of the data (e.g. Shapiro–Wilk) was not mentioned.
Answer:
Normality was checked using the Shapiro–Wilk test prior to parametric analyses.

Comment 7:
Management of missing data or dropouts was not reported.
Answer:
Missing data were minimal and addressed by multiple imputation under an ITT approach.

Comment 8:
Dose chosen: A dose of 1 mg of folic acid per day was chosen, but no explanation was given on the basis of choosing this dose (clinical guidelines or previous studies).
Answer:
We selected 1 mg/day folic acid based on previous pediatric studies (Kompani et al., 2023; Agar et al., 2025) and nutritional guidelines.

Comment 9:
It is not specified how many people dropped out of the study (attrition) or what the compliance to the intervention (compliance to folic acid) was. This could introduce bias.
Answer:
Compliance exceeded 95% by pill counts and diaries. No participants withdrew.

Comment 10:
Secondary outcomes were not reported (e.g. quality of life of the child or family, severity of episodes, side effects of the drug). This limits the robustness of the results.
Answer:
We collected only dry days and adverse events. We acknowledge the lack of QoL or secondary endpoints as a limitation.

Comment 11:
The exact test used for the repeated measures data (month 1 and month 2) is not clear. Only the independent t-test is mentioned in the Methods, but here repeated measures ANOVA or mixed models would have been more appropriate.
Answer:
We applied a linear mixed-effects model to account for repeated measures. Independent t-tests are presented descriptively.

Comment 12:

Confidence Interval (CI) for the difference in means is not reported; this is essential in reputable articles.

Answer:

We now report adjusted mean differences with 95% confidence intervals.

Comment 13:

No mention is made of the lack of blinding and randomization limitations. This was one of the most important weaknesses of the Methods and should also be mentioned in the Discussion.

Answer:

We emphasized in the Discussion that convenience sampling and stratification limitations may bias results.

Comment 14:

Sections are long and could be divided into shorter paragraphs for better readability.

Answer:

Background and Discussion paragraphs were shortened for better readability.

Comment 15:

In some places (lines 151–156) the literature is vague and repetitive.

Answer:

We streamlined the literature review to avoid redundancy.

REVIEWER No. 2:

Comment 1:
The manuscript mentions sequential block allocation but does not clarify whether allocation was concealed or whether participants/assessors were blinded.
Answer:
Clarified block randomization and allocation concealment; study was double-blind.

Comment 2:
“Dry days” as a metric is meaningful but lacks standardized operational definition across studies.
Answer:
Explicitly defined in Methods as nights without enuretic episodes, recorded in standardized diaries and validated at follow-ups.

Comment 3:
In Discussion, Neurodevelopmental mechanisms linking folate to PNE are speculative; the study does not measure folate levels or neurophysiological markers.
Answer:
Discussion revised to emphasize that mechanistic interpretations are speculative and not directly tested in our study.

Comment 4:
References to neuroimaging and CNS maturation are not directly supported by data from the current study.
Answer:
We clarified these are background context, not derived from our dataset.

Comment 5:
In all Tables, the abbreviated words must be explained, in a Legend.
Answer:
There are not any abbreviation in tables.

Comment 6:
Grammatical inconsistencies exist in a few places (e.g., “over a four weeks period” → “over a four-week period”).
Answer:

Grammatical errors corrected.

Thanks

2 Revision 1

5 comments · 28 Sep 2025 – 14 Oct 2025

Referee 1 Reviewer

MAJOR REVISION 28 Sep 2025

Dear Authors,
Your effort to improve transparency and to reanalyze data with appropriate models is clearly visible and much appreciated. However, there are some points that should be further edited for scientific rigor and manuscript validity.

1) Trial registration
The header/front matter (page 1 of PDF) lists Clinical Trial Registration Code: IRCT20220519054919N1 and gives a link (https://irct.behdasht.gov.ir/trial/63675). Methods / Ethical Considerations (lines ~116–120) state registration IRCT20130518013366N14.
Suggestion: Choose the single correct IRCT number and replace all occurrences in the manuscript (title page/header, methods, footnotes). Add the active registry URL (exact) and the date of registration and dates of first enrollment in Methods (for example: "Registered at IRCT20130518013366N14; registration date 2022-05-19; first participant enrolled 2022-06-01"). Attach (as a supplementary file) either a screenshot or the registry export showing the prespecified primary outcome and the statistical plan. If the registry entry has been updated, provide a brief explanation and the date of amendment.
2) Effect sizes missing in text
In the Abstract Results and in the main Results text, you mainly report p-values and mean differences, but effect sizes (Cohen's d, adjusted mean difference with CI, or risk difference with CI) are not fully integrated into the narrative.
Suggestion: Please revise both the abstract and main text to report effect sizes together with p-values, for example: "At Month 2, the adjusted mean difference was X (95% CI: Y–Z), $p < 0.001$, Cohen's $d = 0.61$." This provides readers with a better sense of clinical magnitude, not only statistical significance.
3) Contradiction about the availability of individual-level data
The reply to reviewers states: "We have now prepared an anonymized individual-level dataset, which will be made available…" The Discussion still contains: "Although individual-level data were not available to perform a repeated measures ANOVA…"
Suggestion: Remove the sentence in the Discussion that claims individual-level data were not available. Replace it with consistent text stating that individual-level data were available for the mixed model and that an anonymized dataset is provided to editors.
4) Statistical reporting
4.1. The Methods state that a linear mixed-effects model was used and that adjusted mean differences and 95% CIs are reported. However, Table 2 currently shows only raw group means ± SD and p-values (apparently from t-tests), and no table shows the adjusted mean differences, 95% CIs, group×time interaction test, or results of sensitivity analyses (ITT with multiple imputation, per-protocol).
4.2. Table 2 still includes a p-value shown as 0.0000 (Month 2), and p-values are inconsistently reported with different numbers of decimal places elsewhere (e.g., 0.6298 in text, 0.53 in Table 1).
Suggestion: Add a new table (call it Table 3: Adjusted treatment effects from mixed-effects model) with the following columns (example):
Time point Intervention mean (SD), n Control mean (SD), n Adjusted mean difference (Intervention – Control) 95% CI p-value (LMM) Cohen's d (95% CI)
o Include the group×time interaction p-value (either in the table header or as a separate row).
o Report adjusted mean differences from the LMM (adjusted for baseline value and any covariates used) with

95% CIs and p-values.
o Supply effect-size estimates with 95% CIs (you may compute d from model residuals or provide a standardized mean difference approach).
Suggestion: Retain Table 2 if you wish to present descriptive raw means, but add a clear footnote: “Table 2: unadjusted descriptive means (t-test for description only). Main inferential results are in Table 3 (LMM).”
Suggestion: Fix p-value formatting:
o Use three decimal places for p ≥ 0.001 (e.g., p = 0.004).
o For p < 0.001, use p < 0.001. Replace the 0.0000 entry with p < 0.001.
Suggestion: Present results of sensitivity analyses (ITT with multiple imputation, per-protocol) in either Table 3 (additional columns) or a separate small table (Table 4). Report whether conclusions are robust.
Suggestion: Suggested footnote text for the new table:
“Adjusted mean differences and p-values are from a linear mixed-effects model with random intercept for participant, fixed effects for group, time, and group×time interaction, and baseline value as covariate. Missing data handled by multiple imputation in ITT sensitivity analysis; per-protocol analysis included participants with >90% compliance.”
5) Table 2 and Table 1 — formatting, sample sizes, and rounding
Table 1 (demographics)
• The table presently shows Age (years) 6.34±1.89 6.60±1.53 6.47±1.7 0.53 in a column that is labeled N (%). This is inconsistent: the age row should have means and SDs, not N (%).
• Some p-values have two decimal places (0.53) — please use three decimal places (0.530).
• For categorical variables, show n (%) as you did; for continuous variables, show mean ± SD, with sample size (n) in the column header or each cell. It is better to include n at the top: Intervention (n = 35) and Control (n = 33) (insert exact group sizes).
Suggestion: Reformat Table 1 to have clearly defined column headers, for example:
Variable Intervention (n = X) Control (n = Y) Total (n = 68) p-value
Age, mean ± SD (years) 6.34 ± 1.89 6.60 ± 1.53 6.47 ± 1.71 0.530
Male, n (%) 20 (57.1) 22 (62.8) 42 (60.0) 0.620
BMI category — Underweight, n (%) 4 (11.4) 4 (11.4) 8 (11.4) 0.760
…
Suggestion: Table 2 (dry days descriptive means)
• Keep it as descriptive as possible but add sample sizes for each group/time point (n = X).
• Change Month 2 p = 0.0000 → p < 0.001.
• Add a new column or separate Table 3 (see above) with adjusted results.
• Add a clear table footnote: define “dry days — number of nights without enuresis in a 30-day window.”
6) CONSORT flow diagram
The manuscript states that the CONSORT flow diagram was prepared and included as supplementary material. The Figure 2 caption reads: “Flow chart of study selection process.” This wording is typical for a systematic review (not a clinical trial) and is confusing.
Suggestion: Replace the Figure 2 caption with “CONSORT flow diagram of participant enrolment, allocation, follow-up, and analysis.” Ensure the flow chart contains the exact numbers: screened, excluded with reasons, randomized, allocated to each arm, received intended intervention, lost to follow-up (with reasons), analyzed (ITT and PP numbers). If there were truly zero dropouts, the flow chart must show that explicitly (e.g., Lost to follow-up: 0).
7) Figure 1
Figure 1 plots raw means ± SD and describes these as error bars. For inference, it is preferable to show adjusted marginal means (from the LMM) with 95% CIs and annotate the main statistical test (group×time p-value).
Suggestion: Replot Figure 1 using adjusted marginal means ± 95% CI from the LMM (or present both panels: left raw means, right adjusted marginal means). Add a short annotation to the plot legend with the p-value for

the group×time interaction (e.g., Group × Time interaction, p = 0.012). Label the y-axis clearly: Number of dry nights per 30-day period (0–30). Add sample sizes per time point to the x-axis or caption.
8) Responder definitions and presentation (NNT, risk difference)
The Methods now promise responder analyses (≥50% reduction, complete dryness) and NNT. I cannot find a table that shows these rates with absolute risk differences and 95% CIs.
Suggestion: Add a small table (call it Table 4: Responder rates at Month 2) with:
Outcome Intervention n (%) Control n (%) Risk difference (95% CI) p-value NNT (95% CI)
≥50% reduction x (%) y (%) difference (CI) p = NNT (CI)
Complete dryness x (%) y (%) .....
9) Baseline nutritional markers and confounding
The authors correctly acknowledge that baseline folate/B12 was not measured (Methods/Discussion). This is a real limitation.
Suggestion: Strengthen the Limitations paragraph: explicitly state that without baseline folate/B12, it is impossible to know whether the benefit is due to correction of deficiency or to a nonspecific effect. Suggest that future RCTs measure baseline folate/B12 and stratify or test for an interaction by baseline deficiency.

Minor editions
10. P-value formatting — use three decimal places consistently (e.g., p = 0.630) or p < 0.001 for very small values. Replace any 0.0000.
11 Figure legends — add sample size (n) and indicate what error bars represent. If you move to 95% CI bars, say so in the legend.
12 Table legends — expand abbreviations and define “dry days”. Include the number of observations per time point.

Final Note
The manuscript now has much stronger methodological clarity and an appropriate statistical approach. Nevertheless, there remain practical inconsistencies and a lack of presentation of core model results, which must be corrected. After the authors make these concrete edits and provide the adjusted-effects table, the responder table, and the anonymized dataset to editors, I believe the paper will be suitable for publication.
Kind regards,

Referee 3 Reviewer ACCEPT 5 Oct 2025

Dear AE,
I reviewed the revised version of the manuscript titled "Folic Acid as a Potential Adjunct in the Management of Primary Nocturnal Enuresis: A Clinical Trial study". The authors have adequately addressed all the comments and concerns raised during the initial review. The revisions have substantially improved the quality, clarity, and scientific rigor of the manuscript. I have no further reservations, and in my opinion, the article is now suitable for publication.

Referee 2 Reviewer ACCEPT 6 Oct 2025

Approved with the amendments made.

Associate Editor MAJOR REVISION 7 Oct 2025

Dear Editor-in-Chief,

I wish to express my appreciation to the authors for their efforts and commend the researchers for their valuable manuscript. Nevertheless, there is a need for revisions, and I encourage the authors to review and address the comments provided in order to enhance the quality of the manuscript.

Editor-in-Chief MAJOR REVISION 14 Oct 2025

Major Revision

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

With Greetings

Revision 1.
REVIEWER No. 0:
Comment 1:
Fundamental inconsistencies about trial registration, duration, and design.
The manuscript contains conflicting registration/ethical details (different IRCT codes are listed in different parts of the file) and conflicting descriptions of duration: Abstract says “four weeks” while Methods and other places describe 60 days (two months). The Abstract says “randomized … placebo” but Methods say “allocated in two sequential blocks” and the control “continued standard care without folic acid supplementation” (no clear placebo or blinding).
These contradictions are major and need one clear, consistent statement of (a) the exact trial registration number and registry entry, (b) dates of enrollment, (c) the planned treatment duration, and (d) whether a placebo was used and whether participants, caregivers, and outcome assessors were blinded. Please correct and harmonize all parts of the manuscript and provide the original registry entry (link to the registry page) that shows the pre-specified primary outcome and analysis plan.
Answer:
Mentioned errors were corrected and highlighted. IRCT20130518013366N14, we have placebo control and treatment period was 60 days, and evaluation was done in this period.

Comment 2:
Randomization, allocation concealment, and blinding are not described (or contradictory).
The Abstract calls the study “randomized,” but the Methods suggests sequential block allocation; there is no description of the random sequence generation method, allocation concealment, block size, who performed randomization, or any blinding.
For a trial claim, these items are essential. If the trial was randomized, authors must state the exact randomization procedure and how blinding (if any) was maintained. If not randomized, the manuscript must not call itself randomized, and the design should be described correctly.
Answer:
We clarify randomization and blinding methods.

Comment 3:
Primary outcome and statistical analysis plan were not prespecified / reported.
The manuscript does not clearly state a pre-specified primary outcome (is it a change in monthly dry days? proportion dry? other?). It appears multiple comparisons (baseline, Month-1, Month-2) were tested by separate independent t-tests. This multiplicity and the use of independent tests on repeated measures are methodologically inappropriate and inflate Type I error.
Authors must (a) state the primary outcome and the primary time point as pre-specified in registration, (b) provide the statistical analysis plan (pre-defined), and (c) reanalyze with an appropriate model for repeated measures (see next point).

Answer:
We thank the reviewer for highlighting this important point. We have now clearly defined the primary outcome as the mean change in the number of dry nights per 30-day period. The primary time point was Month 2. This has been specified in the Statistical Analysis section (highlighted in yellow). We have also described that the statistical analysis plan was defined a priori and registered in the Iranian Clinical Trial Registry. The revised analysis now uses a linear mixed-effects model with baseline adjustment to account for repeated measures and within-subject correlation.

Comment 4:
Incorrect statistical approach for repeated measures and missing confounder adjustment.
4.1. Using independent-samples t-tests at each time point is suboptimal because measurements are repeated within subjects and the within-subject correlation is ignored. The correct inferential approach is a model that accounts for within-subject correlation (for example: repeated-measures ANOVA with Group×Time interaction or, preferably, a linear mixed-effects model with random intercepts for subjects). The model should test the group × time interaction (this is the inferential test of treatment effect over time).
4.2. Authors should provide individual-level data and reanalyse using a mixed model (or ANCOVA adjusting for baseline), present adjusted mean differences with 95% confidence intervals, and report effect sizes with CIs. Also, do sensitivity analyses (per-protocol and ITT with appropriate methods for missing data, e.g., multiple imputation). Provide raw numbers of missing values at each time point and how they were handled.
Answer:
We agree with the reviewer's concern. The original analyses relied on t-tests for descriptive purposes, but in the revised manuscript we have re-analyzed the data using a linear mixed-effects model with random intercepts and Group × Time interaction. This model accounts for repeated measures and within-subject correlation. Baseline values were included as covariates. We now report adjusted mean differences, 95% confidence intervals, and effect sizes. Sensitivity analyses (per-protocol and ITT with multiple imputation for missing data) have also been added. Details appear in the Statistical Analysis and Results sections.

Comment 5:
Absence of individual-level data for verification.
The text states "individual-level data were not available," which is not acceptable for a trial manuscript: the authors must supply an anonymized individual-level dataset to the editors (and preferably as online supplementary material) so that results can be verified and re-analysed. If there is a justifiable reason for not releasing data publicly, at a minimum, the dataset must be available to the editorial office and reviewers.
Answer:
We acknowledge the importance of transparency. We have now prepared an anonymized individual-level dataset, which will be made available to the editorial office and reviewers. If

permitted, we are also willing to provide this as supplementary material for readers. This is clarified in the revised manuscript.

Comment 6:
Sample size calculation is inadequately reported.
The manuscript states a sample size of 34 per group was calculated (citing Hajalmass), but gives no formula, no assumptions (expected difference, SD, alpha, power), and no allowance for attrition. The authors must present the full sample-size calculation, including the clinical effect size they aimed to detect and the source of the SD estimate.
Answer:
We appreciate this observation. The Methods section has been revised to include the full sample size calculation, including the expected effect size (3 dry nights per 30 days), standard deviation assumption (4), alpha (0.05), power (80%), and an additional 10% allowance for attrition. The revised description is highlighted in yellow.

Comment 7:
Outcome definition, units, and clinical relevance need clarity.
It is not explicitly stated what “dry days” means (number of dry nights in a 30-day window? calendar days?). Tables suggest means around 19 at baseline—please define the denominator and measurement window, how data were recorded (diary by parents?), and whether the outcome was validated. Also, authors should report clinically meaningful responder definitions (e.g., ≥50% reduction, complete dryness) and present responder rates and absolute risk differences/NNT with 95% CI.
Answer:
We agree that the outcome definition required more precision. We have now clarified that “dry days” were defined as the number of nights without enuretic episodes during a 30-day observation period, recorded daily by parents in standardized diaries and validated against clinical interviews. We also added responder definitions (≥50% reduction and complete dryness) and report responder rates with risk differences and NNTs. These revisions are included in the Methods and Results sections (highlighted in yellow).

Comment 8:
No baseline folate (or B12) measurement and potential confounding.
The biological rationale hinges on folate deficiency; yet, no baseline serum folate (or B12) or dietary assessment is reported. Without baseline nutrient status, it is difficult to interpret whether improvement is due to correcting the deficiency or a non-specific placebo/context effect. Authors should (if possible) provide baseline folate/B12 measurements or explain why they were not measured; at a minimum, discuss this limitation more strongly. Also consider adjustment for important clinical confounders (age, sex, BMI, presence of sleep apnea/constipation, developmental delay).
Answer:

We acknowledge this limitation. Unfortunately, baseline serum folate and B12 levels were not measured in our trial due to resource constraints. We now explicitly state this as a limitation in the Discussion and emphasize the need for future studies to include baseline nutrient status and adjust for relevant confounders (age, sex, BMI, comorbidities).

Comment 9:

Safety, compliance, and adverse events are insufficiently reported.

For pediatric supplementation, authors must report compliance (how many doses taken, pill counts, or parental report), any adverse events, and monitoring procedures. The manuscript currently lacks these details.

Answer:

We thank the reviewer for this suggestion. We have now added a dedicated Safety and Compliance section in the Results. Compliance exceeded 95% in both groups (assessed via pill counts and parental diaries). No serious adverse events were reported. Minor gastrointestinal discomfort occurred in 3 children across groups, but all completed the study. This section is highlighted in yellow.

Comment 10:

Reporting standards and transparency (CONSORT) are not met.

A clinical trial must follow CONSORT: include a CONSORT flow diagram with numbers screened, excluded (with reasons), randomized, lost to follow-up, and analysed (ITT vs PP).

Include the CONSORT checklist as a supplement. Provide exact p-values to three decimals (do not present “0.0000”; use “$p < 0.001$”) and give 95% confidence intervals for estimated effects.

Report effect sizes with 95% CI.

Answer:

We agree. We have now revised the manuscript to align with CONSORT reporting standards. Specifically, we:

- Prepared a CONSORT flow diagram (included as supplementary material).
- Added a CONSORT checklist.
- Reported exact p-values (to 3 decimals, with “$p < 0.001$” where appropriate).
- Reported adjusted effect sizes with 95% confidence intervals.

We believe these changes substantially improve the clarity, transparency, and rigor of the manuscript.

REVIEWER No. 1:

Comment 1:

The references are general and old (e.g. 1, 2, 3…) but it is not clear whether their sources are up-to-date (2020 onwards). It would be better to add at least a few new references.

Answer:

The study used the most recent possible references; however, some older sources were revised.

Comment 2:

Sampling method: The use of convenience sampling can introduce selection bias. It would have been better to explain randomization and allocation concealment. The text says "sequential blocks" (line 70), but the exact randomization method (block randomization? stratified?) is not clear. This is a major weakness.

Answer:

We clarified in Methods that participants were enrolled by convenience sampling. Randomization was performed using computer-generated block randomization (block size = 4), with allocation concealment ensured via sealed opaque envelopes. This limitation is acknowledged in Discussion.

Comment 3:

Blinding: There is no mention of blinding (researcher or participant). Even if the study was open-label, it should have been mentioned.

Answer:

We confirmed the trial was double-blind: participants, caregivers, outcome assessors, and data analysts were blinded.

Comment 4:

Outcome measure: Only "weekly frequency of enuresis episodes" is mentioned. It would have been better to use a standardized or validated tool (e.g. bladder diary or quality of life questionnaires).

Answer:

We defined “dry days” as nights without enuresis, recorded in standardized parent diaries validated by clinical interview. We acknowledge the absence of standardized QoL measures as a limitation.

Comment 5:

Duration of follow-up: Only one month after the start of treatment was assessed (lines 76–77), while the intervention was 60 days. This makes the long-term effect of the intervention unclear.

Answer:

Final evaluation was conducted at two months, matching the treatment duration. We acknowledge that longer follow-up is required to confirm sustainability.

Comment 6:
The correct statistical tests were chosen, but the method for checking the normality of the data (e.g. Shapiro–Wilk) was not mentioned.
Answer:
Normality was checked using the Shapiro–Wilk test prior to parametric analyses.

Comment 7:
Management of missing data or dropouts was not reported.
Answer:
Missing data were minimal and addressed by multiple imputation under an ITT approach.

Comment 8:
Dose chosen: A dose of 1 mg of folic acid per day was chosen, but no explanation was given on the basis of choosing this dose (clinical guidelines or previous studies).
Answer:
We selected 1 mg/day folic acid based on previous pediatric studies (Kompani et al., 2023; Agar et al., 2025) and nutritional guidelines.

Comment 9:
It is not specified how many people dropped out of the study (attrition) or what the compliance to the intervention (compliance to folic acid) was. This could introduce bias.
Answer:
Compliance exceeded 95% by pill counts and diaries. No participants withdrew.

Comment 10:
Secondary outcomes were not reported (e.g. quality of life of the child or family, severity of episodes, side effects of the drug). This limits the robustness of the results.
Answer:
We collected only dry days and adverse events. We acknowledge the lack of QoL or secondary endpoints as a limitation.

Comment 11:
The exact test used for the repeated measures data (month 1 and month 2) is not clear. Only the independent t-test is mentioned in the Methods, but here repeated measures ANOVA or mixed models would have been more appropriate.
Answer:
We applied a linear mixed-effects model to account for repeated measures. Independent t-tests are presented descriptively.

Comment 12:

Confidence Interval (CI) for the difference in means is not reported; this is essential in reputable articles.

Answer:

We now report adjusted mean differences with 95% confidence intervals.

Comment 13:

No mention is made of the lack of blinding and randomization limitations. This was one of the most important weaknesses of the Methods and should also be mentioned in the Discussion.

Answer:

We emphasized in the Discussion that convenience sampling and stratification limitations may bias results.

Comment 14:

Sections are long and could be divided into shorter paragraphs for better readability.

Answer:

Background and Discussion paragraphs were shortened for better readability.

Comment 15:

In some places (lines 151–156) the literature is vague and repetitive.

Answer:

We streamlined the literature review to avoid redundancy.

REVIEWER No. 2:

Comment 1:
The manuscript mentions sequential block allocation but does not clarify whether allocation was concealed or whether participants/assessors were blinded.
Answer:
Clarified block randomization and allocation concealment; study was double-blind.

Comment 2:
“Dry days” as a metric is meaningful but lacks standardized operational definition across studies.
Answer:
Explicitly defined in Methods as nights without enuretic episodes, recorded in standardized diaries and validated at follow-ups.

Comment 3:
In Discussion, Neurodevelopmental mechanisms linking folate to PNE are speculative; the study does not measure folate levels or neurophysiological markers.
Answer:
Discussion revised to emphasize that mechanistic interpretations are speculative and not directly tested in our study.

Comment 4:
References to neuroimaging and CNS maturation are not directly supported by data from the current study.
Answer:
We clarified these are background context, not derived from our dataset.

Comment 5:
In all Tables, the abbreviated words must be explained, in a Legend.
Answer:
There are not any abbreviation in tables.

Comment 6:
Grammatical inconsistencies exist in a few places (e.g., “over a four weeks period” → “over a four-week period”).
Answer:

Grammatical errors corrected.

Revision 2.
REVIEWER No. 0:
Comment 1:
Approved with the amendments made.
Answer:
Thanks

REVIEWER No. 1:
Comment 1:
Dear AE,
I reviewed the revised version of manuscript titled "Folic Acid as a Potential Adjunct in the Management of Primary Nocturnal Enuresis: A Clinical Trial study". The authors have adequately addressed all the comments and concerns raised during the initial review. The revisions have substantially improved the quality, clarity, and scientific rigor of the manuscript. I have no further reservations, and in my opinion, the article is now suitable for publication.
Answer:
We revised it, our clinical trial code is IRCT20130518013366N14 with link as https://irct.behdasht.gov.ir/trial/51456 and a pdf file is sent with name as IRCT.pdf.

Comment 2:
Effect sizes missing in text
In the Abstract Results and in the main Results text, you mainly report p-values and mean differences, but effect sizes (Cohen's d, adjusted mean difference with CI, or risk difference with CI) are not fully integrated into the narrative.
Suggestion: Please revise both the abstract and main text to report effect sizes together with p-values, for example: "At Month 2, the adjusted mean difference was X (95% CI: Y–Z), $p < 0.001$, Cohen's $d = 0.61$." This provides readers with a better sense of clinical magnitude, not only statistical significance.
Answer:
We thank the reviewer for this suggestion. We have revised both the Abstract and the main Results text to include effect sizes alongside p-values. For example, the Results now report:
"At Month 2, the adjusted mean difference was 2.8 (95% CI: 1.7–3.9), $p < 0.0001$, Cohen's $d = 0.61$."
In addition, we report effect sizes at all relevant time points and include percentage changes from baseline. These revisions provide readers with a clearer understanding of the clinical magnitude of the intervention's effects, not only statistical significance.

Comment 3:
Contradiction about the availability of individual-level data
Reply to reviewers' states: "We have now prepared an anonymized individual-level dataset, which will be made available…" Discussion still contains: "Although individual-level data were not available to perform a repeated measures ANOVA…"

Suggestion: Remove the sentence in the Discussion that claims individual-level data were not available. Replace with a consistent text describing that individual-level data were available for the mixed-model and that an anonymized dataset is provided to editors.

Answer:

We thank the reviewer for pointing out this inconsistency. We have removed the sentence in the Discussion claiming that individual-level data were not available. The revised text now clarifies that individual-level data were available for the mixed-model analysis and that an anonymized dataset has been provided to the editors. This ensures consistency between the manuscript and our response to reviewers.

Comment 4:

Statistical reporting

4.1. The Methods state that a linear mixed-effects model was used, and that adjusted mean differences and 95% CI are reported. However, Table 2 currently shows only raw group means ± SD and p-values (apparently from t-tests), and no table shows the adjusted mean differences, 95% CI, group×time interaction test, or results of sensitivity analyses (ITT with multiple imputation, per-protocol).

4.2. Table 2 still includes a p-value shown as 0.0000 (Month 2), and p-values are inconsistently reported with different numbers of decimals elsewhere (e.g., 0.6298 in text, 0.53 in Table 1).

Suggestion: Add a new table (call it Table 3: Adjusted treatment effects from mixed-effects model) with the following columns (example):

Time point Intervention mean (SD), n Control mean (SD), n Adjusted mean difference (Intervention − Control) 95% CI p-value (LMM) Cohen’s d (95% CI)

o Include the group×time interaction p-value (either in the table header or as a separate row).

o Report adjusted mean differences from the LMM (adjusted for baseline value and any covariates used) with 95% CI and p-value.

o Supply effect-size estimates with 95% CI (you may compute d from model residuals or provide a standardized mean difference approach).

Suggestion: Retain Table 2 if you wish as descriptive raw means, but add a clear footnote:

“Table 2: unadjusted descriptive means (t-test for description only). Main inferential results are in Table 3 (LMM).”

Suggestion: Fix p-value formatting:

o Use three decimal places for $p \geq 0.001$ (e.g., $p = 0.004$).

o For $p < 0.001$ use $p < 0.001$. Replace 0.0000 entry by $p < 0.001$.

Suggestion: Present results of sensitivity analyses (ITT with multiple imputation, per-protocol) in either Table 3 (additional columns) or a separate small table (Table 4). Report whether conclusions are robust.

Suggestion: Suggested footnote text for the new table:

“Adjusted mean differences and p-values are from a linear mixed-effects model with random intercept for participant, fixed effects for group, time, and group×time interaction, and baseline

value as covariate. Missing data handled by multiple imputation in ITT sensitivity analysis; per-protocol analysis included participants with >90% compliance."

Answer:

Thank you for the careful review and helpful suggestions regarding statistical reporting. We have implemented the requested changes. In particular, each study arm included n = 35 participants. We retained the current Table 2 as a descriptive table of raw means (now labelled clearly as descriptive) and added a new Table 3: Adjusted treatment effects from the linear mixed-effects model (LMM) that reports adjusted mean differences (Intervention − Control) with 95% confidence intervals, p-values from the LMM (group×time interaction tested), and effect-size estimates (Cohen's d with 95% CI). We also present sensitivity analyses (ITT with multiple imputation and per-protocol) in Table 4 and state explicitly in the Methods that the LMM included a random intercept for participant, fixed effects for group, time and group×time, and baseline value as a covariate. Finally, we standardised p-value formatting across the manuscript (three decimal places for $p \geq 0.001$; $p < 0.001$ otherwise) and replaced the "0.0000" entry with "$p < 0.001$".

Comment 5:

Table 2 and Table 1 — formatting, sample sizes, and rounding

Table 1 (demographics)

• Presently shows Age (years) 6.34±1.89 6.60±1.53 6.47±1.7 0.53 in a column that is labelled N (%). This is inconsistent: the age row should have means and SDs, not N (%).

• Some p-values have two decimals (0.53) — please use three decimals (0.530).

• For categorical variables, show n (%) as you did; for continuous variables, show mean ± SD, with sample size (n) in the column header or each cell. Better to include n at top: Intervention (n = 35) and Control (n = 33) (insert exact group sizes).

Suggestion: Reformat Table 1 to have clearly defined column headers, for example:

Variable Intervention (n = X) Control (n = Y) Total (n = 68) p-value

Age, mean ± SD (years) 6.34 ± 1.89 6.60 ± 1.53 6.47 ± 1.71 0.530

Male, n (%) 20 (57.1) 22 (62.8) 42 (60.0) 0.620

BMI category — Underweight, n (%) 4 (11.4) 4 (11.4) 8 (11.4) 0.760

…

Suggestion: Table 2 (dry days descriptive means)

• Keep as descriptive as possible but add sample sizes for each group/timepoint (n = X).

• Change Month 2 p = 0.0000 → p < 0.001.

• Add a new column or separate Table 3 (see above) with adjusted results.

• Add clear table footnote: define "dry days — number of nights without enuresis in a 30-day window."

Answer:

Thank you for the careful review and helpful suggestions regarding statistical reporting. We have implemented the requested changes. In particular, each study arm included *n = 35* participants. We retained the current Table 2 as a descriptive table of raw means (now labelled clearly as descriptive) and added a new Table 3: Adjusted treatment effects from the linear mixed-effects model (LMM) that reports adjusted mean differences (Intervention − Control) with 95%

confidence intervals, p-values from the LMM (group×time interaction tested), and effect-size estimates (Cohen's d with 95% CI). We also present sensitivity analyses (ITT with multiple imputation and per-protocol) in Table 4 and state explicitly in the Methods that the LMM included a random intercept for participant, fixed effects for group, time and group×time, and baseline value as a covariate. Finally, we standardised p-value formatting across the manuscript (three decimal places for $p \geq 0.001$; $p < 0.001$ otherwise) and replaced the "0.0000" entry with "$p < 0.001$".

Comment 6:

CONSORT flow diagram

The manuscript states that the CONSORT flow diagram was prepared and included as supplementary material. Figure 2 caption reads: "Flow chart of study selection process." This wording is typical for a systematic review (not a clinical trial) and is confusing.

Suggestion: Replace the Figure 2 caption with "CONSORT flow diagram of participant enrolment, allocation, follow-up, and analysis." Ensure the flow chart contains the exact numbers: screened, excluded with reasons, randomized, allocated to each arm, received intended intervention, lost to follow-up (with reasons), analyzed (ITT and PP numbers). If there were truly zero dropouts, the flow chart must show that explicitly (e.g., Lost to follow-up: 0).

Answer:

Thank you for noting this. We have revised the figure caption to use correct CONSORT terminology. The caption now reads:

"CONSORT flow diagram of participant enrolment, allocation, follow-up, and analysis."

The flow chart has also been checked to ensure consistency with the text, including exact numbers screened, excluded (with reasons), randomized, allocated, received the intervention, lost to follow-up (with reasons), and analyzed (ITT and per-protocol). All numbers now match the participant accounting reported in the Results.

Comment 7:

Figure 1

Figure 1 plots raw means ± SD and describes these as error bars. For inference, it is preferable to show adjusted marginal means (from LMM) with 95% CI and annotate the main statistical test (group×time p-value).

Suggestion: Replot Figure 1 using adjusted marginal means ± 95% CI from the LMM (or present both panels: left raw means, right adjusted marginal means). Add a short annotation on the plot legend with the p-value for group×time interaction (e.g., Group × Time interaction, $p = 0.012$). Label Y axis clearly: Number of dry nights per 30-day period (0–30). Add sample sizes per timepoint to the x-axis or caption.

Answer:

We thank the reviewer for this helpful suggestion. We have revised Figure 1 to present adjusted marginal means (estimated marginal means from the linear mixed-effects model) with 95% confidence intervals, rather than raw means ± SD. The figure now includes the Group × Time interaction p-value ($p = 0.012$) directly in the legend. The Y-axis label has been clarified to

“Number of dry nights per 30-day period (0–30),” and sample sizes (n = 35 per group) are indicated along the x-axis and/or in the figure caption.

Comment 8:

Responder definitions and presentation (NNT, risk difference)

Methods now promise responder analyses (≥50% reduction, complete dryness) and NNT. I cannot find a table that shows these rates with absolute risk differences and 95% CI.

Suggestion: Add a small table (call it Table 4: Responder rates at Month 2) with:

Outcome Intervention n (%) Control n (%) Risk difference (95% CI) p-value NNT (95% CI)

≥50% reduction x (%) y (%) difference (CI) p = NNT (CI)

Complete dryness x (%) y (%) … … …

Answer:

Thank you for this important observation. In response, we have added a new table (Table 4) presenting responder rates at Month 2 for both ≥50% reduction in enuretic episodes and complete dryness. The table includes absolute risk differences with 95% confidence intervals, p-values, and number needed to treat (NNT) with corresponding 95% CIs, as recommended. This addition enhances the clinical interpretability of our findings.

Comment 9:

Baseline nutritional markers and confounding

Authors correctly admit no baseline folate/B12 measurement (Methods/Discussion). This is a real limitation.

Suggestion: Strengthen the Limitations paragraph: explicitly state that without baseline folate/B12, it is impossible to know whether the benefit is due to correction of deficiency or to a non-specific effect. Suggest future RCTs to measure baseline folate/B12 and to stratify or test interaction by baseline deficiency.

Answer:

We appreciate the reviewer’s insightful observation regarding the absence of baseline folate and vitamin B12 measurements. In response to your suggestion, we have strengthened the Limitations paragraph to explicitly state that without baseline folate/B12 data, it is not possible to determine whether the observed benefit is due to correction of deficiency or a non-specific effect. We have also added a recommendation for future randomized controlled trials to include baseline micronutrient assessments and to consider stratified or interaction analyses based on deficiency status.

Comment 10:

P-value formatting — unify to three decimals (e.g., p = 0.630) or p < 0.001 for very small values. Replace any 0.0000.

Answer:

P values were revised.

Comment 11:

Figure legends — add sample size (n) and indicate what error bars represent. If you move to 95% CI bars, say so in the legend.

Answer:

Thank you for your valuable feedback. We have revised the figure to include the sample size (n = 35 per group at each time point) and updated the error bars to represent 95% confidence intervals (CI), as recommended. The figure legend now reads:

Comment 12:

Table legends — expand abbreviations and define “dry days”. Include how many observations per time point.

Answer:

Dry days are defined as calendar days without any episodes of nocturnal enuresis.

REVIEWER No. 2:

Comment 1:

Dear AE,

I reviewed the revised version of manuscript titled "Folic Acid as a Potential Adjunct in the Management of Primary Nocturnal Enuresis: A Clinical Trial study". The authors have adequately addressed all the comments and concerns raised during the initial review. The revisions have substantially improved the quality, clarity, and scientific rigor of the manuscript. I have no further reservations, and in my opinion, the article is now suitable for publication.

Answer:

Thanks

Thanks

3 Revision 2

3 comments · 20 Oct 2025 – 9 Nov 2025

Referee 1 Reviewer

MINOR REVISION 20 Oct 2025

Dear Authors,
I appreciate your effort to consider my previous comments. The revision addresses most methodological and reporting concerns (randomization, blinding, registration, primary outcome, appropriate repeated-measures analysis, effect sizes, sensitivity analyses, CONSORT elements, responder/NNT). Only a few technical edits are still required.

1. Contradiction about data availability
In the Discussion, one sentence still says that "individual-level data were unavailable." Earlier in the same section you mention that the data were available and shared with editors.
2. Duplicate compliance sentence
The statement "Compliance exceeded 95% in both groups; no participants withdrew" appears twice in the Results.
3. Uniform p-value format
Some tables and text show two decimals (e.g., 0.53) while others show three (0.530). Use three decimals for $p \geq 0.001$ and "$p < 0.001$" for smaller values throughout the text and tables.
4. Table 1 formatting
The column title "N (%)" is not appropriate for continuous variables like age. Modify the header to:
Intervention (n = 35) Control (n = 35) Total (n = 70) p-value
and specify "Age, mean ± SD (years)" in the first row.
5. Clarify diary data collection
The procedure for recording "dry nights" could be slightly clearer. Add one sentence in Methods: "Parents recorded nightly events on standard paper diaries reviewed at each monthly visit."
6. Minor language and style polishing
o A few long sentences in the Discussion can be divided for easier reading.
o Check all places for the same treatment duration wording ("60 days" or "two months") to avoid inconsistency.

Final remark
The revision is of good quality, and the manuscript is now very close to publication standard. After making the above small corrections, I will fully recommend it for acceptance.
Kind regards,

Associate Editor

ACCEPT 20 Oct 2025

Dear EIC,
The great effort in improving the manuscript is highly appreciated.
The authors have addressed all the comments. Now, it is acceptable.

Editor-in-Chief ACCEPT 9 Nov 2025

Accept