Title: Effects of dietary supplementation with a standardized aqueous extract of Terminalia chebula fruit (AyuFlex®) on joint mobility, comfort, and functional capacity in healthy overweight subjects: a randomized placebo-controlled clinical trial

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Author’s response to reviews:

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EFFECTS OF DIETARY SUPPLEMENTATION WITH A STANDARDIZED AQUEOUS EXTRACT OF TERMINALIA CHEBULA FRUIT (AYUFLEX®) ON JOINT MOBILITY, COMFORT, AND FUNCTIONAL CAPACITY IN HEALTHY OVERWEIGHT SUBJECTS: A RANDOMIZED PLACEBO-CONTROLLED CLINICAL TRIAL


BMC Complementary and Alternative Medicine
Reviewer #1

The authors appreciate your time and report. Thank you for the positive comment surrounding the writing and preparation of our manuscript. We hope to further improve it through the review process.

Please see our responses below to suggestions, points and comments to improve the manuscript.

[Comment]
line 22 pg 6 correct "chromatographm";
line 17 pg 5 write "LBP" with all words in its complete form for the first time;

RESPONSE:
Noted and corrected in the updated draft. Thank you.

[Comment]
line 56 pg 5  specify the % value of the flavonoids, such as tannins and shaccarides;

RESPONSE:
Noted and thank you for the request. We have added more detail in the revised draft, with accurate information from certificates of analyses from the research sponsor as follows:

“The tannins are most prevalent at 32%-56%, and include gallic acid, ellagic acid, chebulic acid, chebulinic acid, punicalagin, and tannic acid. The flavonoids include quercetin, catechin, and kaempferol. Saccharides are present at 6%-9%, and include D-glucose, D-fructose, and saccharose. Quinic acid at 1.5%, and shikimic acid at 2% are the prevalent fruit acids [1, 2, 3, 19].
Chemical analysis of AyuFlex®, a commercially available standardized aqueous extract of T. chebula fruit (Natreon Inc., New Jersey, USA) (AF), indicates a phytochemical profile that includes ≥39% low molecular weight hydrolysable tannins, with ≥15% chebulinic and ≥12% chebulagic acid, and flavonoids at 5.2%, and D-glucose + D-fructose sugars at 6.1% as analyzed by HPLC (see Fig. 1).”

Also, we’ve included detailed information within the METHODS section, page 13, line 60:

“HPLC analysis of commercial batch AYF-110315 of the test product AyuFlex®, a standardized aqueous extract of T. chebula fruit (commercial product from Natreon Inc., New Jersey, USA) (AF) was confirmed to contain 58% tannins, with 33.8% chebulinic acid, 9.4% chebulagic acid, 9.4% gallic acid, 3.4% elagic acid, 5.2% total flavonoids, 6.1% sugars as D-glucose and D-fructose. Both AF and Placebo capsules included microcrystalline cellulose, croscarmellose sodium (24 mg), silicon dioxide (6 mg) and magnesium stearate (6 mg) as excipients. Placebo capsules contained microcrystalline cellulose at 400 mg, while AF 250 mg capsules contained 300 mg of microcrystalline cellulose and AF 500 mg capsules contained 109 mg microcrystalline cellulose.”

[Comment]

lines 28,29 pg 7 you wrote "The protocol was reviewed, and all procedures approved by an independent, FDA- audited,Institutional Review Board…..", but the board was indipendent or institutional?

RESPONSE:

It is independent. We have also added “external” to help clarify. Here is a more detailed description from this central IRB: IntegReview IRB is an external independent institutional review board that provides ethical, scientific and regulatory review to approve, and conduct periodic review of biomedical, medical device, social, educational and behavioral research involving human subjects in the United States, Latin America, Japan and may collaborate with a Canadian-based IRB that conducts Canadian site reviews. complies with the regulations as defined in the United States Food and Drug Administration (FDA), Code of Federal Regulations,
Title 21, Parts 50, 54, 56, 312 and 812, International Conference on Harmonisation (ICH) Guidelines for Good Clinical Practices, E6, the Department of Health and Human Services (DHHS) regulations as identified in the Code of Federal Regulations, Title 45, Part 46, other regulations as applicable, as well as local and state laws.

*The authors feel it is written appropriately in the current draft, but we are open to different verbiage or further detail such as provided above.

[Comment]
- I think that the extract composition, characterization and product formulation/composition could be better describe. Was it a commercial product?

RESPONSE:
Thank you for the suggestion. Yes, this is a commercial product. This comment is now addressed per above in both the INTRODUCTION and METHODS sections of the revised manuscript.

[Comment]
- How did you choose the daily dose?

RESPONSE:
The authors addressed the prior studies utilizing this same commercial product toward the end of the INTRODUCTION section, pages 6-7, lines 27-60. However, we’ve added the following to clarify further.

“Additional double-blind, placebo-controlled trials have shown that oral administration of T. chebula fruit extract (AF) at 250 mg and 500 mg twice daily successfully reduced pain and joint discomfort compared to placebo, with statistically significant improvements in pain threshold
force and time, and pain tolerance force and time (P < 0.001) [20] and reductions in mWOMAC and knee swelling index, and visual analog scale scores of pain, stiffness, and disability [21].

[Comment]
- Describe the placebo content.

RESPONSE:
This has now been addressed on Page 13 and 14, as stated above:

“Both AF and Placebo capsules included microcrystalline cellulose, croscarmellose sodium (24 mg), silicon dioxide (6 mg) and magnesium stearate (6 mg) as excipients. Placebo capsules contained microcrystalline cellulose at 400 mg, while AF 250 mg capsules contained 300 mg of microcrystalline cellulose and AF 500 mg capsules contained 109 mg microcrystalline cellulose.”

Reviewer #2

The authors appreciate your time and report. Thank you for the positive comment surrounding the writing and preparation of our manuscript. We hope to further improve it through the review process.

Please see our responses below to suggestions, points and comments to improve the manuscript.
1. The authors mention that they collected dietary intake and physical activity data of subjects. However, they did not report the results anywhere in the manuscript.

RESPONSE:

Thank you for your comment. We analyzed 3-day diet records and performed a 24-hr diet duplication prior to subjects reporting for their study visits for biological specimen and data collection. We agree that it is important to report these data, and the authors have incorporated energy intake, carbohydrate, protein and fat intake at baseline and over course of the study on page 19.

2. Statistical analysis should be adjusted for some important variables such as age, body mass index, physical activity and energy intake of subjects.

RESPONSE:

Thank you for your comment. We chose to run an ANCOVA using the respective variables of interested (at baseline) but did not use other variables in the covariance matrix (e.g. BMI, physical activity, etc) because those variables were not different between the groups at baseline (see Table 3). Nonetheless, we conducted a few additional ad-hoc analyses using these variables and the results were no different. Therefore, we are confident in our initial analysis and interpretation.
Abstract

1. The authors should give rationale behind selection of T. chebula fruit for joint mobility, comfort, and functional capacity in introduction.

RESPONSE:

Thank you for the suggestion. The authors included rationale in the introduction on pages 5 and 6, but have now including an additional statement as part of the ABSTRACT section.

“…The fruit of Terminalia chebula has been used extensively in various traditional health systems for different ailments, with additional preclinical and clinical data demonstrating antioxidant and anti-inflammatory potential…”

Introduction

1. The authors address some other studies about T. chebula fruit extract. However, the gap of other studies has not been mentioned.
RESPONSE:

Thank you for your comment. We have added another transitional statement at the end of the Introduction section describing some of the limitations or gaps in prior/other studies. Below is the added statement:

“However, prior to this present study, the AF product had yet to be studied for dose-response effects in healthy subjects without advanced musculoskeletal pathology, and under a more rigorous design with a placebo lead-in.”

[Comment]

Methods

1. The authors mention that “the subjects were ranked according to bodyweight, placed into block groups of 3 and .....”. If they mean stratified randomization, the categories for body weight should be mentioned.

RESPONSE:

Thank you for your comment. We did not use strict cut offs for blocking subjects according to body weight.

[Comment]

2. Regarding to high frequency intake of multivitamin in America, please provide information on this dietary supplement.

RESPONSE:

Thank you for the comment. We are unsure how to address, as there is no reliable data on prevalence or rate of use of this particular commercial product in the United States. The authors believe there may be some unverified data available from industry trade organizations on sales of
multivitamin and dietary supplements in general, as well as some sales data from surveys and private brands on use of botanical extracts or herbal products.

[Comment]

3. The authors mention that "A total of 166 potential subjects were contacted for participation". However, the sampling method is not clear. How these subjects identified to be contact?

RESPONSE:

Thank you for your comment. These facts have been added to the manuscript on page 12.

We recruited our subjects from the population of northeast Ohio using flyers, word-of-mouth, and our database of previous studies (which contains ~ 10,000 subjects). Subjects were initially contacted by telephone and email prior to being interviewed and screened.

[Comment]

4. The criteria for overweight is not provided.

RESPONSE:

Thank you for your comment. We used the National Institutes of Health (NIH) classification system of BMI to determine whether subjects were overweight or not (i.e. BMI = 25-29.9). This fact has been added to the manuscript on page 9.

[Comment]

5. In inclusion criteria, the following criteria should be placed in exclusion criteria: Non-smoker-Subjects with a history of knee or hip joint replacement surgery, or any hip or back pain which
interferes with walking or exercise testing utilized throughout the study- No knee joint discomfort at rest.

RESPONSE:

Thank you for the suggested edits. The authors have made the changes on pages 10 & 11.

[Comment]

6. Inclusion criteria in the text and table are not the same.

RESPONSE:

Thank you again for the comment. The authors have made changes on pages 9, 10 & 11 to verify that the text and table now match with the accurate and actual inclusion (and exclusion) criteria.

[Comment]

7. The age range in Abstract and Table 2 is not the same.

RESPONSE:

Thank you for the comment. We have implemented the changes in the table such that the Abstract and Table 2 are now consistent.

[Comment]

8. The authors mention that "subjects were enrolled, and randomized into one of three parallel groups to participate in the study". However, the method used to generate the random allocation sequence has not been explained.
RESPONSE:

Thank you for your comment. The website: https://www.randomizer.org was used to generate a random allocation sequence into groups. This fact has been added to the manuscript on page 7 of the METHODS section.

[Comment]

9. The placebo content and its production agent are not mentioned.

RESPONSE:

Thank you for your comment. This has now been addressed per responses to reviewer 1 above, and the detail has now been added to the revised draft of the manuscript on page 14.

[Comment]

10. It is not clear why the authors did not calculate body mass index instead of just weight?

RESPONSE:

Thank you for noting this oversight. The authors have now simply calculated BMI from the weight and height. This has been added to the manuscript on page 19, Table 3. beneath “Weight.”
11. It is not clear if the blood sampling was in fasting state.

**RESPONSE:**

Thank you for bringing this to our attention. The authors have clarified this point on page 8. Blood was collected after a 10 hour overnight fast.

12. Please provide reasons for using multiple criteria for assessing outcome variables.

**RESPONSE:**

Thank you again for your comment. It is unclear to us what the Reviewer is actually asking for, but our ANCOVA was used to assess between-group differences, while a delta score analysis was used to detect within-group differences over time. In our experience over the past 20 years, this is a customary approach to data analysis in this type of study.

13. Please provide references for methods of measuring inflammation biomarkers.

**RESPONSE:**

Thank you for the suggestion. The authors have included references for methodology utilized, and also renumbered references accordingly after adding new references #26 and #27 on Page 17.
Results

1. Please spell out some abbreviations such as LBP in the text and PBO in tables

RESPONSE:

Thank you for the comment and suggestion. The authors have already assigned LBP in a previous comment from Reviewer #1, but we have now spelled out PBO as part of the caption on each table where PBO appears.

RESPONSE: Thank you for the review. We have attempted to address all comments provided by both reviewers and the editors. We feel the improvements have strengthened our manuscript.