Reviewer’s report

Title: The Effect of Total Anthocyanin-base Standardized (Cornus mas L.) Fruit Extract on Liver Function, Tumor Necrosis Factor α, Malondialdehyde, and Adiponectin in Patients with Nonalcoholic Fatty Liver: A study protocol for a double-blind randomized clinical trial.

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Reviewer: Peter Curtis

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Review of Manuscript Number: NUTJ-D-19-00006
Title: The Effect of Total Anthocyanin-base Standardized (Cornus mas L.) Fruit Extract on Liver Function, Tumor Necrosis Factor α, Malondialdehyde, and Adiponectin in Patients with Non-alcoholic Fatty Liver: A study protocol for a double-blind randomized clinical trial submitted to: Nutrition Journal

The manuscript by Sangsefidi et al., describes the pre-intervention protocol for a human RCT intervention with Cornelian cherry fruit extract in patients with NAFLD. The study aims to conduct a 12 week parallel study to determine the effectiveness of the study treatment on a number of primary (identified in the introduction as liver function, TNF-alpha, MDA and adiponectin) and secondary endpoints of relevance to NAFLD. The subject area is of potential clinical interest and the interaction between flavonoids and NAFLD remains an understudied area in human RCTs.

Hereafter, please find comments relating to the study design and information presented within the manuscript.

Main comments:

1. The clarity and consistency of which variables are primary endpoints could be improved. In the clinical trials directory (IRCT20180419039359N1), 8 well defined primary endpoints are identified, yet the manuscript is somewhat vague at times. In the abstract, and introduction, 'liver function' TNF-alpha, MDA and adiponectin are specifically mentioned, but it isn't until the 'primary outcomes' section where ALT, AST, CK-18M30, hepatic steatosis and liver fibrosis are mentioned. The actual markers of function (and injury? i.e. CK-18M30) should be explicitly identified in the abstract and introduction. Confirmation, in the introduction, that the study was powered on ALT, would also be of interest to the reader. As would some broad reference to the wide range of secondary outcomes assessed (i.e. parameters of insulin resistance and lipid / lipoprotein status).

2. Whilst the introduction mentions many associations between liver function, NAFLD and anthocyanin (in vitro / in vivo studies), there is no explicit rationale presented why ALT, AST, TNF-alph, MDA (which isn't mentioned at all), adiponectin, CK-18 M30 are primary endpoints.
Presumably, this is due to being a combination of markers of liver health, injury and fibrosis, inflammation, glucose and fatty acid regulation and oxidative stress - but the mechanism and rationale why these are selected in a NAFLD population, over insulin resistance, as an example of a secondary marker, is currently not clear enough.

3. The evidence to suggest that the primary markers are modifiable over 12-weeks should be referenced in the introduction (if this data exists).

4. Can the authors use a more accessible descriptor for the active treatment arm? There is a repetitive use of the description 'total anthocyanin-base standardized cornelian cherry fruit extract' which breaks the flow of the text in multiple places. It is correct to describe in longhand in the descriptor section of the interventions, but once described, perhaps paraphrase to Cornelian cherry extract, or anthocyanin extract, or cherry-anthocyanin extract (or similar).

5. A more detailed description of the likely intervention end-products is required (if this can be estimated). For cherry-anthocyanin extract, are other flavonoid / phenolic compounds expected to be in the extract? or is this a purified anthocyanin? (if so, it would be good to make that explicit). How will the intervention material be presented and consumed; i.e. an encapsulated powder? Liquid? Syrup? How many capsules etc. per day? Regarding the placebo, the description is that it will be 'similar' - in what way? Macro/micronutrient composition? What comparative material is being used as a placebo filler?

6. Regarding the balancing of the treatment groups during randomisation - the authors have identified that age and sex will be used. Considering the primary endpoints, can the authors justify why BMI (likely to be highly correlated with adiponectin) and a marker of NAFLD progression (i.e. fatty liver grade 1, 2, 3) are not being included to ensure the groups are similar?

7. Can the authors please clarify the cut-off being used for treatment adherence? In various places (e.g. study population section, intervention section), the text seems to suggest that anyone consuming more than >20% will be deemed as compliant. I'm assuming this is incorrect, as a high quality RCT would be benchmarking treatment adherence at >80-85%. Perhaps the authors meant that non-compliance of greater than 20% would mean an exclusion (i.e. <80% intake). An RCT with only >20% adherence to treatment would be unpublishable.

8. A lack of biological sampling to confirm compliance to intervention, through i.e. anthocyanin / phenolic metabolite analysis (either in urinary or serum analyses) is a limitation and may reduce the impact of the study outputs.

Hereafter are additional minor comments for specific sections:

Abstract
* Confirm that the extract group will receive it for 12 weeks (it currently only identified that for the placebo).
* The word 'the beneficial effects' should be removed and replaced with 'the effect' - the research question should be objective, without a suggestion of preconceived benefits.
Introduction
* Give greater detail of how this study will address the limitations identified; e.g. rather than just saying that steatosis and fibrosis were previously identified using imprecise methods - give details of the validated and precise methods that are being used in this study.

Methods
* Mention the word 'parallel' in the study design description.
* What do you mean by 'authenticity'? that they were genuinely cherries? Or that their anthocyanin content will be confirmed?

Preparation of placebo
* First sentence, delete everything before 'A placebo of similar extract..' The current start to the sentence isn't about the placebo.

Study population
* Will a nationally / internationally recognised validated method be used to define NAFLD by the clinicians? if so, please refer to this, to provide objectivity that all clinicians would judge the same patients are having NAFLD.
* Regarding consumption of medications that affect liver function - presumably, this includes over the counter medications such as painkillers? Can this be included in the description.
* Are drugs being monitored during the study too - currently, it is written that the exclusion only counts for one month before the study.

Ethical considerations and trial registration
* Informed consent is taken after protocol review, and presumably before any screening. Figure 1 is incorrect in this respect, and the 'consent forms' text should be moved to before 'screening for eligible participants'

Randomization:
* The text currently says 'using a random method.' - this should say 'using a method of randomisation' as the method isn't random. Can the authors add the reference for the Random Allocation Software?

Intervention:
* The text says 'The control group will also receive a placebo with the same dose for 12 weeks' - again, I think this is an error and could conceivably say, 'The control group will also receive a placebo, matched for the same weight, appearance, taste and colour (but without any anthocyanin) for 12 weeks'

Data collection:
* Is a 3d food diary sufficient? Food intake records are notoriously poor indicators of habitual intake (but relatively accurate of monitored 3d intake) and perhaps another measure (FFQ?) might give a more rounded assessment of typical intakes?
* Is there any monitoring of change in weight / adiposity? (which would conceivably affect adiponectin, and almost every other cardiometabolic endpoint).
* Is Waist and hip measurement possible in this population? This may provide relevant measure of central adiposity for a NAFLD population.

Primary outcomes
* The elastography and fibroscan method should be described somewhere *(methods?), including a reference to the equipment used and the validated protocol used.

Level of interest
Please indicate how interesting you found the manuscript:

An article of importance in its field

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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