

## Reviewer Comments.

There is an urgent need for adequate treatment for the growing NAFLD and hepatocellular carcinoma. This manuscript reveal a natural product extract that could be beneficial.

Simple editing:

All "et al. " in the manuscript should be in italics.

Please search the entire manuscript and change.

Line 52. Replace restore with treat and prevent

Line 72. in the south of China, as well as in India and south east Asia.

Line 96. Five standard (lower case s)

Line 107: Delete The primary antibodies and replace simply with Antibodies to

Line 115: Change concentrated to concentrating

Line 112: Define TQPE as *Trapa quadrispinosa* pericarp extract. Also, the name of the plant must always be in italics.

Line 196. Please change dominated to predominant

Line 239. Define HOMA-IR. Homeostatic Model Assessment of Insulin Resistance.

Line 321. Please change to used in other clinical trials.

Line 332. ..waste, that are usually discarded

### Questions that need to be answered:

Line 153. How do we know that the mice continue to eat the high fat diet after beginning treatment? It is possible that they lost their appetite and ate less. Was the amount of food eaten (or left in food tray) measured?

Line 153. How ere these doses selected. Normally one selects does based on blood levels and scaled down from toxic limits

In the introduction the authors should note if the pericarp of TQ is used in China as natural medicine for liver disease. Is this used what led the authors to identify the active components?

Also, there are other *Trapa* species (such as *T. japonica* and others. Is the activity specific to TQ?

Was there any plasma pharmacokinetic analysis on the TQPE conducted? Steady state plasma levels are essential. It will also show which of the components are actually absorbed. Of the several constituents of the TQPE it is possible that in the intestinal tract some of them may be converted and it is also likely that only one or more components are actually absorbed. What is the oral bioavailability of each component? This is important and needs to be determined prior to publishing this manuscript. The authors may be surprised that only one or more components are needed and also that there may be conversion in the gut.

Line 245. Was there a dose response between the 15 and 30 mg/kg doses tested in each of the parameters?

It would be helpful to have a Figure of the proposed NAFLD signaling pathways. I note the pathway as the last Figure. That Figure also needs clarification. There are several up and down arrows. Which of these are related to the TQPE treatment. It is not clear to me.