REPLY TO COMMENTS

Reviewer's code: 00504391

Major comments

1. No major changes in liver ultrastructure are found between the NDEA- and the Lycopene +NDEA treated groups. Please discuss thoroughly.

Reply: Fig.1.D and E clearly showed the surface morphology of the liver tissue of NDEA treated group. Scanning electron micrograph depicts discernible necrotic tumor nodules of irregular shape, uncontrolled cell density, wretched histo-architecture, rounding of hepatocytes which clarify the presence of undifferentiated HCC.

On the other hand Fig.1.F, G and H showed SEM micrographs of liver tissue from LycT + NDEA group. On comparing these micrographs from control group it was concluded that at 24th week after NDEA treatment there was increased cell density, trabecular pattern of hepatocytes and high percentage of apoptotic bodies, indicating well differentiated HCC. But lycopene pretreatment has significantly reduced the severity caused by NDEA treatment when compared with the micrograph of NDEA group.

Similarly, the description of TEM micrographs has been elaborated in result section.

As advised by learned reviewer, for the ease of understanding the comparison in the ultrastructure of NDEA and LycT + NDEA, a quantification of important parameters has been presented in tabulated form (Table 2 and 3).

2. Based on the lycopene amount provided to the animals, how much lycopene should a person take to prevent HCC? Which are the potential side-effects of such concentrations?

Reply: So far, it is in infancy to estimate the lycopene dosage for human to treat HCC. Because in the animal study, initially study was designed to find the lethal dose and then trials were made with the reduce doses and finally the minimum concentration dose with high efficacy was chosen i.e. 5 mg/kg. We have also done some dose related studies in our pilot experiments (Gupta et al., 2013) and also considering the literature available. There are many commercially available combinations containing lycopene as their principle active component, but the concentration is different in different formulation. Although lycopene is a strong antioxidant but at high concentration its pro-oxidative nature has also been reported. Also, like any other herbal drug, excess of lycopene consumption may shows devastating liver and kidney complications.

3. Definitely, the language should be revised by experts.

Reply: As advised the language has been revised.

4. Figure 4 is confusing. The title indicates that it is the mRNA expression but the Table in the graph indicates "Protein" Please clarify.

Reply: The necessary corrections has been made in the table, protein is replaced with mRNA.

5. Did they observe the ultrastructural changes in all the NDEA treated animals? No analysis exists of such observations. The authors should show a quantitative analysis of the SEM and TEM observations.

Reply: As suggested the observation of ultrastructural changes in NDEA and LycT + NDEA has been quantified and presented in tabulated form (Table 2 and 3).

6. Based on the gene and protein expression changes observed, the authors should add a new Figure showing the potential mechanism of HCC prevention by lycopene.

Reply: Graphical representation of overall mechanism of lycopene is introduced at the end of the article.

Minor comments

2. In some cases, spaces are missing between values and units.

Reply: Necessary corrections have been made.

3. Description of Panel G is missing in Figures 1 and 2, please make it clear.

Reply: Actually Fig.1.F-H included the description of Fig. 1.G. But now it has been replaced with Fig.1. (F, G and H). Similarly, amendment has been done in Fig. 2.

4. In the Results Section, the authors should specify from the very beginning of the corresponding paragraph if they refer to either mRNA or protein expression.

Reply: As suggested, the line introducing the mRNA or protein expression has been incorporated in the result section at appropriate location.

5. Discussion. The authors mention that "Electron micrographs (SEM and TEM) of liver biopsies from different groups provided a real picture of 3-D in-vivo tissue modulations…" They are not showing real pictures in vivo, they are actually removing the tissues from the animal. Please rephrase.

Reply: The phrase has been removed and revised as suggested.

Reviewer's code: 03538959

1. The cancer type of the animal model should be specified in the Highlights.

Reply: As suggested by the learned reviewer, the necessary corrections have been made.

2. The author needs to display and detail the HE images of HCC occurrence.

Reply: The display and detail about the H&E images has already made in the previous publication (Gupta et al., 2013).

3. How did the author get the conclusion that lycopene modulates cellular proliferation without MTT in vitro?

Reply: In the current study, various cell proliferation associated genes has been studied at mRNA and protein level. On the basis of the observation LycT + NDEA treatment has significantly ameliorated the increased mRNA and protein level upon NDEA treatment. As such no *in-vitro* studies has been designed for this study, but can be planned for future studies.

4. Why the author chose the ELISA method to detect the proteins in liver tissues?

Reply: The study was designed to quantify the mRNA and protein expression of targeted genes during carcinogenesis and its amelioration by LycT. Thus, for mRNA we included reverse transcriptase PCR and for protein quantification we included ELISA for its high sensitivity and accuracy in quantifying the protein level.

5. The title should be corrected clear and concise.

Reply: Title can be reframed as "Lycopene modulates various structural and molecular aspects during NDEA induced hepatocellular carcinoma"