Reviewer's report

Title: Astragalus polysaccharides inhibit P-glycoprotein efflux pump function and decrease its protein expression in H22 hepatoma cells in vitro

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Reviewer: Ashish Mehta

Reviewer's report:

Tian and coworkers suggest that addition of Astragalus polysaccharides (APS) could sensitize the effect of anti-cancer drugs by inhibiting P-glycoprotein efflux pump function and decreasing protein expression in H22 hepatoma cells. However, the study is weak and there are too many anomalies in the presented results that hamper the acceptance of the manuscript. It seems that the authors were very casual in their approach while conceptualizing the study.

1. Result section; In table 2 authors demonstrate effects of sensitizing cells with APS, why did the authors not use doses higher than 500mg/mL to evaluate if inhibition rates could be higher as in the case of DDP.

2. Does N.S stand for “Normal Saline”. Please add the same in figure legend for table 2.

3. Table 2, It is not clear how did the authors calculate the percentage inhibition. Looking at the formula provided in the material and methods the resultant values don’t match. For eg. for APS 0.8mg/ml, (1-0.71(test)/3.918 (control))x100 = (1-0.181)x100= 8.19% . This value is nowhere close to -0.67% as reported by the authors. Author need to clarify?

4. Since the IC50 for APS was calculated as 278 mg/mL, did the authors observe reduction in cell viability with the addition of 500mg/mL of APS. What was the loss of percentage of live cells. How did the authors normalize the following difference when calculating the % inhibition as well as evaluating results?

5. It would be better if the authors convert table 3 in a graphical format for better understanding of the data. Secondly, some of the data does not look statistically significant as indicated by the authors. Authors need to re-evaluate the same. Eg, VCR (control vs 0.8mg APS) does not seem to significant.

6. Looking at the MTT results (OD values) in table 2, it seems that addition of 0.8mg/mL of APS could significantly reduce cell proliferation (0.71) as compared to controls (3.9). How would authors then co-relate this significant loss of cells especially when added other drugs like ADM etc to calculate IC50 values. It seems that there is already more than 90% cell loss after addition of APS?

7. What concentration of anti-cancer drugs did the authors use in not mentioned in the table 3? How did the authors select the following doses too is not mentioned?

8. Figure 1, Authors state that compared to the controls, the RFP fluorescence
shifted to the left. However, this is not clear in the figure. The values presented by the author in table 4 for control vs RFP group at 24h do not match with the figure?

9. For gene expression studies, what was used to normalize the test data is not shown. Are the values presented relative to control or RFP group?

10. Table 1 can be omitted and the doses can be included in the material and method section.

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests