Reviewer's report

Title: Frankincense oil derived from Boswellia carteri induces tumor cell specific cytotoxicity

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Reviewer: Katarina Hostanska

Reviewer's report:

Authors Frank et al investigated the effect of frankincense oil on the viability of normal bladder urothelial UROtsa cells and on one bladder carcinoma J82 cells. They used XTT assay for the measuring of the cytotoxic effect and in addition, apoptotic cell death was investigated by DNA fragmentation and the effect of frankincense oil on gene expression was identified with microarray analysis.

The question posed by the authors was the different cytotoxicity between normal and cancer bladder cells. There are some queries with the methods. An effect of only one bladder cancer cell line is not enough to postulate specificity of an agent. Further, as shown in Figure 2 (which presents means and SE, not SD), the mean standard error of experiments with UROtsa cells is very large in comparison to J82 cells. Why? To evaluate statistical differences the authors should use ANOVA with adequate post hoc test instead only t-test. Usually, for the differences between single cell lines their IC50 values are determinate. The effect of frankincense oil was achieved after 24h treatment of cells and normalized to untreated values. There is a discrepancy between result and conclusion (pages 11 and 12). In the untreated controls the number of viable J82 cells increased 1.62-fold and that of UROtsa cells 2.72-fold. When UROtsa cells were treated with 1:600 dilution of frankincense oil, there was a 1.29-fold change. Please, explain, why is it no change to the 2.72 value by untreated cells? Overall it seems to be a 2-3-fold difference at the concentration of frankincense needed for comparable effect on both cell lines. Therefore a comparison between IC50 will be very important and improving the manuscript. In addition, an influence of culture medium on the effect of frankincense oil in UROtsa cells could not be excluded. It is well known, that different amount of supplemented FCS play a role by the cytotoxic effect of different agents.

Further, authors investigated the induction of apoptosis by frankincense oil in J82 cells by morphological investigation and by DNA fragmentation. They could not find any DNA fragmentation. The apoptosis was determined only morphologically. Second method for the detection of apoptosis will be necessary (e.g. annexinV/PI stain).

The modulation of gene expression on J82 cells was identified by microarray assay. Frankincense oil altered gene products related to transcription factors, proliferation and cell cycle arrest, as well as apoptotic factors. In the relation to the apoptosis, there could be determined both up-regulated pro-apoptotic as well
as anti-apoptotic genes. Therefore a second method for apoptotic cell death determination will be necessary.

Further, there is an unbalance between the results and postulation of the specificity of frankincense oil (title), as well as the conclusion, that frankincense oil might represent an alternative intravesical agent for bladder cancer treatment, is inadequate to the presented results. Experiments on more bladder carcinoma cell lines are required.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests