LETTER TO THE EDITOR

ABOUT THE ARTICLE

"Cytotoxicity Effects Of Ethanolic Extract Of Punica Granatum Var. Pleniflora On Mcf-7 Compared With L929 Cells; Gavanji S., Bakhtari A., Baghshahi H., Badripour N., Hamami Chamgordani Z. (2023). The New Armenian Medical Journal, vol.17(3), p 25-30; https://doi.org/10.56936/18290825-2023.17.3-25".

The presentation of all experimental results in Figures 1 and 2 raises substantial methodological and ethical concerns regarding the integrity and authenticity of the underlying data. The complete absence of any variability measures (SD, SEM, confidence intervals, or replicate information) is already scientifically unacceptable for MTT assays, which are inherently noisy and highly sensitive to plate position, pipetting precision, and environmental conditions. **However, a more serious issue is the *abnormally smooth, perfectly monotonic, and almost geometrically linear pattern* observed in both dose–response curves**. Such uniformity is highly atypical of biological systems and inconsistent with the well-established sigmoidal behavior of cytotoxic dose–response relationships. Real experimental data — even under ideal conditions — invariably exhibit fluctuations, deviations, or scatter due to biological, technical, and procedural variability. The consistently perfect decline across all concentrations, without a single irregularity or deviation, is statistically improbable for genuine multi-replicate MTT data.

The improbably high R^2 values reported for linear regression, coupled with the absence of SD or variance metrics, further exacerbate concerns. Without access to replicate-level absorbance values or any evidence of biological variability, it is impossible to rule out excessive data smoothing, post-hoc curve fitting, selective reporting, or other forms of inappropriate data handling. While this does *not* imply intentional fabrication, the current presentation is inconsistent with expected experimental variability and thus raises red flags regarding the reliability of the dataset.

Also, several substantial miss data, scientific inconsistencies and methodological concerns were identified that may affect the validity and reliability of the reported findings. we summarize the most critical issues below:

1. Major inconsistencies between the abstract, main text, tables, and figures, including contradictory dose–response values and mismatched lethal concentration ranges. The lethal concentration ranges described in the abstract do not match the values reported in the tables, and some dose–response trends are internally inconsistent. Such discrepancies raise concerns about data accuracy and manuscript integrity. Table 2 (Cytotoxic activity in L929 cells) with Figure 2 on survival rate of L929 paper is just reply on MTT/cytotoxicity. The graphical presentation of the cytotoxicity results in Figures 1 and 2 raises serious concerns about the reliability, authenticity, and analytical rigor of the data. First, the survival curves are plotted without any indication of variability—no standard deviation, standard error, confidence interval, or even the number of replicates is provided. For an assay such as MTT, which is inherently prone to intra-assay and inter-assay fluctuations, omission of variance metrics is scientifically unacceptable and prevents any meaningful interpretation of the results. More importantly, the curves exhibit an unnaturally smooth and almost perfectly linear decline across concentrations for both cell lines. Such a pattern is biologically implausible for dose–response behavior, which universally follows a sigmoidal or at least nonlinear pharmacodynamic profile. The absence of scatter, variability, or deviation from a straight line strongly suggests that the data may not represent genuine experimental replicates or that the results have been oversimplified, overprocessed, or manually fitted. This undermines the credibility of the reported IC50 values and the validity of the regression model, particularly given the suspiciously high R^2 values reported without transparency regarding data points.

2. Incorrect IC_{50}/CC_{50} methodology, where linear regression was used instead of a nonlinear sigmoidal model, leading to values that conflict with the reported data.

The authors calculated IC_{50}/CC_{50} values using *linear regression* across the entire concentration range. This method is **scientifically inappropriate** for MTT viability assays, which typically follow a **sigmoidal (4-parameter logistic/Hill) curve**, not a linear one.

Additionally, the reported CC_{50}/IC_{50} values do not match the raw values shown in the tables and charts, indicating potential calculation errors or data mislabeling.

3. Due to the inconsistencies in values, incorrect IC_{50} methodology, missing controls, and unclear replicates, the MTT graphs **do not meet the standards required for reliable interpretation**.

4. Inadequate or inappropriate control groups, including the use of 2% DMSO without validation of non-toxicity, and the absence of a positive control to verify assay performance.

The negative control is reported as **2% DMSO**, which may be cytotoxic in many cell lines.

No justification is provided to confirm that 2% DMSO does not affect cell viability.

A **positive control** (e.g., doxorubicin) is also missing, making it impossible to judge the assay's performance or reliability.

The vehicle control does not match the extraction solvent used (70% ethanol), which is inconsistent with standard cytotoxicity assay practice.

5. Insufficient reporting of replicates and statistical analysis, with unclear n-values, inconsistent p-value

reporting, and missing SD/SEM values.

The manuscript does not clearly state the number of biological or technical replicates used.
Although ANOVA and Tukey tests are mentioned, the statistical framework is incomplete:

- SD/SEM values are missing or insufficient
- p-values and multiple-comparison details are inconsistently reported
- The statistical significance indicators do not match the data shown

6. Unclear MTT assay parameters, including unconventional wavelength measurements and missing details regarding background subtraction.

The manuscript does not clarify:

- The plate reader's configuration
- Whether background subtraction was performed
- Whether solvent absorbance was corrected

This omission limits reproducibility and raises concerns about assay validity.

7. The absorbance was measured at **560 nm**, which deviates from the commonly accepted 570 nm (with a reference wavelength around 630–690 nm).

8. Use of a non-human "normal" cell line (L929) for selectivity comparison with human cancer cells, which limits biological relevance.

The manuscript compares a human cancer cell line (MCF-7) with a **mouse fibroblast line (L929)** as the "normal" control.
Cross-species comparisons are not appropriate for assessing selectivity or therapeutic index.
A human non-tumorigenic breast epithelial line (e.g., **MCF-10A**) would be scientifically appropriate.

9. Use of different concentration ranges for different cell lines without justification, preventing meaningful comparison of cytotoxicity.

MCF-7 cells were tested in one concentration range, while L929 cells were tested in a completely different and higher range without scientific justification.
This approach prevents a valid comparison of cytotoxic selectivity and may bias interpretations.

10. Lack of phytochemical characterization of the extract, preventing reproducibility or mechanistic interpretation.

- No chemical profiling (e.g., HPLC, LC–MS) is provided for the plant extract.
- Without characterizing the active components, the mechanistic interpretation and reproducibility of the study are severely limited.

Given the cumulative weight of these issues, and If these concerns cannot be adequately addressed with transparent documentation, the scientific integrity of the article could be compromised. we submit these points in the interest of maintaining high research standards and ensuring accuracy in the published literature. Thank you for your attention to this matter.

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