Reviewer's report

**Title:** Retama monosperma n-hexane extract induces cell cycle arrest and extrinsic pathway-dependent apoptosis in Jurkat cells

**Version:** 1  **Date:** 11 October 2013

**Reviewer:** Mahmoud ALHOSIN

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In this manuscript, the authors investigated the anti-cancer effects of (Rm-HE) (Retama monosperma hexane extract) on the Jurkat cells. They found that Rm-HE could inhibit the cell viability and cause cell cycle arrest in Jurkat cells. Sub G1 analysis also suggested that Rm-HE also induce apoptosis in Jurkat cells. Authors tried to study the molecular mechanism leading to Rm-HE-induced apoptosis in Jurkat cells.

**Major Compulsory Revisions:**

1. JNK is a key regulator of Fas-L expression, so a time-course effect on JNK phosphorylation in Jurkat cells should be performed to confirm the data shown in Figure 4D.

2. Figure 2D shows that the percentage of early apoptotic cells at 24 h was 2.5%. This data is not in agreement neither with Figure 2A showing that the percentage of SubG1 (apoptotic cells) was 33.05% at 24h or with Figure 3B showing that cell viability was reduced to 40% of control after 24 h of treatment with Rm-HE. The authors need to clarify these controversy findings.

3. The authors mentioned that Rm-HE caused a time-dependent increase in Fas-Ligand expression. This is not clear in the Figure 4C. To confirm this conclusion, the authors should make a statistic analysis for Fas-L blot in Figure 4C (three different experiments).

4. The authors mentioned in this study (in conclusion section) that this work clearly indicates that bioactive components of Rm-HE act either alone or in combination to promote cellular apoptosis. Effect of isolated major compounds (such as #-Linoleic acid, Stigmasterol, and Campesterol) on cell viability or apoptosis in Jurkat cells should be performed to show the anti-proliferative and pro-apoptotic effects of bioactive components of Rm-HE.

5. The authors should improve the quality of western data; some blot is not suitable for publication in the scientific journals (eg. Fig2C, Fig3C Caspase-3). Moreover, the same Tubulin blot has been used in all western blots in this study.

**Minor comments**

In the discussion section, the authors indicated the presence of a previous study
about the anti-leukemic effect of RmHE and cited the reference number [28], this last (Reactive oxygen species contribute to cell killing and P-glycoprotein downregulation by salvicine in multidrug resistant K562/A02 cells. Cancer BiolTher.; 6(11), 1794-9) does not talk about the extract RmHE.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.