Reviewer's report

**Title:** Shikonin inhibits ERK 1/2 phosphorylation at an early stage of adipocyte differentiation in 3T3-L1 cells.

**Version:** 1  **Date:** 13 March 2013

**Reviewer:** Jesil Aranjani

**Reviewer's report:**

**Major Compulsory Revisions**

1. Line 67 says DMEM supplemented with calf serum was used for growing the cells. In line 72 the serum supplementation is shown as FBS. Which is the most suitable serum supplement to be used and why? How can you change the serum supplement?

2. Line 75; “various concentrations of shikonin.” Mention how you prepared the dilutions and what is the solvent used.

3. Line 79 MTT assay Mention the reference of this method. Why the wavelength of 490 was chosen for measurement, the standard method recommends 540nm or 570 nm. Mention the medium used and incubation conditions provided in the study.

4. Description of the MTT assay is not adequate, give a detailed description on cell attachment and treatment with the Shikonin.

5. What is the reference for oil red assay

6. RT-PCR, mention the cell lines used for the study.

**Minor revisions**

1. Use same abbreviations throughout the paper
2. Use italics when writing the botanical names in the abstract as well.

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

**Quality of written English:** Needs some language corrections before being published

**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