Reviewer’s report

Title: Centipede Grass exerts anti-adipogenic activity through inhibition of the AKT signaling pathway

Version: 1 Date: 7 June 2012

Reviewer: Wenbin Shang

Reviewer’s report:

Major Compulsory Revisions

1. In order to exclude that the inhibitory effect of centipede grass extract on adipogenesis might be caused by its cytotoxicity, the authors first assessed that cytotoxic effects of CG in 3T3-L1 cells with MTT and LDH assay and concluded that CG extract did not cause cytotoxicity in both the undifferentiated and differentiated 3T3-L1 cells. However, there is a flaw in the experiment about cell viability. The cells were treated with CG extract, meanwhile the cells were induced with DMII or not. This finally resulted in different ratio of differentiated adipocytes and preadipocytes among the four groups when the MTT and LDH assay were performed. Owing to the possible different activity and amount of the related enzymes between preadipocytes and adipocytes, the viability of cells could not be compared based on the reduced MTT and release of LDH. Additional experiments are needed to confirm the conclusion just in preadipocytes without induction or differentiated adipocytes.

2. In the conclusion, the authors concluded affirmatively that CG inhibited adipocyte differentiation by attenuating the expressions of C/EBP# and PPAR# mediated by suppressing the phosphorylations of AKT and GSK3#. However, no direct evidence in this experiment supports above conclusion, although some previous reports demonstrated the link between AKT signaling and adipogenic transcription factors. It is not excluded other mechanism involved in the inhibitory effect of CG on adipogenic transcription factors. The author should interpret the data more stringently.

Minor Essential Revisions

1. In methods, the differentiation/induction medium (DMII) contains 100 mM insulin. The concentration of insulin was very high, contrasting to that of inducer commonly used in adipocyte differentiation. Is there a mistake in the writing? It is better to explain the provenance of this protocol.

2. The sources of antibodies used in Western blot should be mentioned.

3. In Fig.3 A and B, the categorical label of each bar is obscure.

4. The labels of Y-axes are missing in Fig.2 C.

Discretionary Revisions

It is better to move the data and interpretation of TG content assay to first part of
the result, combined with the data of Oil red staining, both of which describe effect of CG on adipogenesis. So, Fig. 3 C should be changed to Fig. 1 D.

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

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests.