Author's response to reviews

Title: Kaliziri extract upregulates Tyrosinase, TRP-1, TRP-2 and MITF expression in Murine B16 Melanoma cells

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Version: 4 Date: 18 February 2014

Reviewer: Masaru Toriyama

Reviewer's report:

Minor Essential Revisions:

I think the study in your manuscript is very interesting. I can agree that the manuscript would be accepted. But one minor revision is necessary for acceptance.

In "Results and discussion", you described the effect of KZE on tyrosinase activity and melanin synthesis in B16 cells. The result showed that melanin levels were increased by KZE treatment. But the result did not show that KZE activated the enzyme (tyrosinase) directly. If you want to show that KZE activates tyrosinase, you should measure in vitro using the mixture containing L-DOPA, KZE and the extract of B-16 cells including tyrosinase. Or you should use mushroom tyrosinase.
I don't think the in vitro tyrosinase assay is necessary. But you had better revise the paragraph described about the effect of KZE on tyrosinase.

Author's response to reviews:


The effect of KZE on mushroom tyrosinase activity was subsequently investigated in vitro. The results show that at five different concentrations KZE increased the tyrosinase activity in a dose-dependent manner.(The result give in Figure 2.)

2. Correction the writing style of scientific plant names such as Vernonia anthelmintica (L.) Willd. Because Willd. is the short name of the author.

This manuscript has some mistakes and need some spelling correction, such as the wrong word: falconoid (flavonoids; page 3), at at concentration (at concentration; page 11), Uygur (Uyghur; page 3), Uighur (Uyghur; page 13), L-3, 4-dihydroxyphenylalanine (page 5), etc.

It also needs to punctuate after full stop or comma. Some words that use the abbreviation already must be use continuously, for example traditional Uyghur medicine (TUM)

We use professional language editing service to improve the quality of the manuscript.

3. In the introduction: the author must explain more about MITF.

We add the paragraph in the introduction:

MITF is the most important transcription factor involved in the regulation of tyrosinase gene expression, which is involved in the pigmentation, proliferation and survival of melanocytes [19 and 20], thus MITF plays a pivotal role in melanogenesis[21 and 22]. It has been reported to bind to the M-box within the TYR promoter, and thus upregulate TYR gene expression [23].

4. In the materials and methods, preparation of KZE (page 6) recommend to correction:

250g of seeds of Kaliziri (Vernonia anthelmintica (L.) Willd) was soaked in ethanol–water (80:20, v/v) 2.5 L for 1h at room temperature and then the extract was filtered and extracted with 2.5 L of ethanol–water (80:20, v/v) at room temperature the solution filtrate was then evaporated under reduced pressure by using rotary evaporator to obtain the ethanol extract, with the yield being 3.38% w/w of the dry weight of the seeds. (Pharmacopoeia of People's Republic of China, Uyghur Medicine volume) [3].

After being dried KZE, the dark brown powder paste was obtained and then dissolved in PBS. Stock solution of KZE (5mg/ml) was prepared in PBS for downstream
application.

5. In the results: we give more details about positive controls such as 8-MOP in order to compare with the effect of KZE.

Tyrosinase activity was determined by L-DOPA oxidation. Stimulation of tyrosinase activity of B16 cell by KZE at 5-40µg /ml. Melanin content were performed as described in "Materials and methods", B16 cells , the same by KZE at 5-40µg /ml. 8-MOP being positive controls at 50µM.(Result give in Fig.4-5)

6. In abbreviations: recommend to give more details about some abbreviations such as TYR, EP tube.


7. In references: recommend to have only one style of reference writing follows with the journal because the authors mixed many styles of writing.

We use professional language editing service to have only one style of reference writing follows with the journal.

8. Suggest the authors to read the paper:


Authors read the paper:

Authors think different points of our article are:

Firstly, we identified the components by LC-MS/MS from kaliziri extract; secondly, we study on effect of KZE on TRP-1 and TRP-2 protein expression in B16 cells.

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

Quality of written English: Acceptable

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

Reviewer’s report

Title: Kaliziri extract up regulates Tyrosinase, TRP-1, TRP-2 and MITF expression in Murine B16 Melanoma Cells

Version: 1 Date: 19 January 2014

Reviewer: Sukanya Dej-adisai
Reviewer's report: An article of importance in its field
Level of interest: An article of importance in its field
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

Reviewer's report

Title: Kaliziri extract up regulates Tyrosinase, TRP-1, TRP-2 and MITF expression in Murine B16 Melanoma Cells
Version: 1 Date: 19 January 2014
Reviewer: Kuo-Ching Wen

The similar study, alcohol extract of Vernonia anthelmintica seed enhances melanin synthesis has been published by Zhou J on J Ethnopharmacology. (Zhou J, Shang J, Ping F, Zhao G. Alcohol extract from Vernonia anthelmintica (L.) wild seed enhances melanin synthesis through activation of the p38 MAPK signaling pathway in B16F10 cells and primary melanocytes. J Ethnopharmacol. 2012 143:639-47.) The effect of Vernonia anthelmintica on melanin synthesis and its mechanism on tyrosinase, MITF, p38 MAPK and protein kinase A has been studied. The novelty of the manuscript “Kaliziri extract up regulates Tyrosinase, TRP-1 and TRP-2 and MITF expression in Murine B16 Melanoma Cells” is not enough.

Authors think different points of our article are:
Firstly, Preparation of extract from traditional Uyghur medicine (Kaliziri) is used method of Pharmacopoeia of the People’s Republic of China, Uyghur Medicine volume [3]; refer to the method of preparation of Kaliziri injection.
Secondly, we identified the components by LC-MS/MS from kaliziri extract and confirm the main content of extract. This work is not done in the above article.
Thirdly, we study on effect of KZE on TRP-1 and TRP-2 protein expression in B16 cells. This work is not done in the above article too.

Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal
Quality of written English: Not suitable for publication unless extensively edited
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests: I declare that I have no competing interests'