Reviewer's report

Title: Angelica sinensis promotes myotube hypertrophy through PI3K/Akt/mTOR pathway

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Reviewer: David Cameron-Smith

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The present manuscript shows the beneficial effect of Angelica sinensis (SA) in myotube diameter and that this effect is through increased mTOR pathway activation. The paper is well conducted with proper controls being carried for most of the experiments. However the paper should be revised, and only accepted if major concerns stated below are properly addressed or explained.

Major compulsory revisions:

1. Although the study shows that SA increase myotube diameter and that it seems to be mediated via mTOR pathway, it does not address or provide data whether it is direct effect on mTOR pathway or enhancing mTOR pathway by facilitating IGF-1 binding to its receptor. Throughout the serum was used in all treatment media, this it is possible that SA exerts its actions through facilitating the action of the growth factors within this media. Parallel studies in serum free conditions are required before conclusions of actions can be drawn.

2. It is not clear in which media the chemicals were diluted in. It is only stated that “For the treatment, the stocks were diluted in the medium”. Which medium is that, growth media (containing serum) or serum free media?

3. To induce differentiation, C2C12 are usually treated with horse serum (HS), it seems that it was not used in this study. How the cells were differentiated?

4. In the Abstract the claim that AS could be used in ergogenic aid for elderly and athlete is an overestimation of the results presented. The results from the study may at most indicate a potentially ergogenic effect on muscle, but further suggestion of which population it could aid is not possible to estimate at this point.

4. Discussion needs further revision by the authors. The parallel made with exercise activation of Akt is inconsistent with the evidence that actions of exercise on mTOR signalling are Akt independent mechanism. Moreover the suggestion that Akt phosphorylation at different time points are due to de novo synthesis of growth factors for the 15 min time point and at 45 min due to autocrine/paracrine loop of IGF-1 are both groundless. Additionally, 45 min of treatment is not likely to increase expression of Akt protein as stated as a possible mechanism (“The second peak that appeared 45 min after AS treatment was possibly mediated by the autocrine/paracrine loop of IGF-1, because the
expression of Akt (…) has been known to be increased by AS treat”).

Minor essential revisions:

1. The western blot membranes were stripped an reprobed using the same secondary antibody, did the authors tested for stripping efficiency? This is important because the phosphorylation data were normalized by the specific total proteins which when proteins are not efficiently removed may affect the densitometry of the second probing (total Akt and mTOR). In the methods it is stated that b-actin was also verified. Do phosphorylation of Akt and mTOR remain the same when normalized by b-actin? The use of a different molecular weight protein (as b-actin) might be useful to clarify this.

2. Is the SD in the Pre bar representing variation within different experiments or different membranes? Please state.

3. Figure 4A: it is missing ‘l’ ‘e’ on Relative at the figure legend.

Discretionary revisions

1. Line 298: “de novo” should be in italic.

2. Keywords: Akt and mTOR is already on the title, no need to add them in the keywords.

Level of interest: An article of limited interest

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.