Reviewer's report

Title: Chotosan ameliorates cognitive and emotional deficits in an animal model of type 2 diabetes: possible involvement of cholinergic and VEGF/PDGF mechanisms in the brain

Version: 2 Date: 27 July 2012

Reviewer: Masayuki Hiramatsu

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The paper entitled: "Chotosan ameliorates cognitive and emotional deficits in an animal model of type 2 diabetes: Possible involvement of cholinergic and VEGF/PDGF mechanisms in the brain", shows that Chotosan (CTS) may have neuroprotective effects on cholinergic and VEGF/PDGF systems involving cognitive deficits in db/db mice. The manuscript is clear and well-focused on the subject. However, I have several concerns about this paper, as follows.

Major Compulsory Revisions:

The Student-Newman-Keuls test compares all pairs of means following one-way ANOVA. Although the whole point of multiple comparison post tests is to keep the chance of a Type I error in any comparison to 5%, in fact, the Newman-Keuls test does not do this. In some cases, the chance of a Type I error can be greater than 5%. (The Newman-Keuls test works fine with three groups; the increase in Type I error occurs only with four or more groups.) Further, the Student-Newman-Keuls test requires equal “N” and normality. The paper by Curran-Everett and Benos (2007) does not show which statistical analysis of the data should be used.

Discretionary Revisions:

The relative expression levels of ChAT, M1 AChR, M3 AChR, and M5 AChR were about the same in db/db control mice and both compounds, CTS and THA, reversed these expressions. When the ACh concentration decreases, the numbers of AChR might increase due to a compensatory mechanism. How do these compounds work with these parameters? Do they work as neuroprotectants too? Is the neuronal mechanism involving the anxiolytic effect resistant? If CTS works as a neuroprotectant, this compound should ameliorate any other model with cognitive deficits. Does it have beneficial effects because diabetes induces some neuronal damage in the brain? You might discuss these points.

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the
statistics.

Declaration of competing interests:
I declare that I have no competing interests.