Investigating the effect of herbs in reducing glucose toxicity in *Caenorhabditis elegans*

Tan Wei Da Darrin, Benz Chong Tze Jun, Kieron Oh Hong Rui

Group 1-22

Abstract

Diabetes mellitus is a common worldwide disease, with severe complications such as heart disease, stroke, kidney disease and nerve damage. Due to the severity of these diseases and infections that may follow, Diabetes mellitus could end up being fatal. Treatment for Diabetes mellitus could also incur high costs, which could bring more problems to the person. This study aims to investigate the effect of increasing glucose concentration on the survival and locomotion of *C. elegans*, and whether herbs were able to reverse glucose toxicity in *C. elegans*. Prepared bacterial preculture was placed on plates of NGM agar with differing glucose concentrations, and in the absence and presence of ginseng extract. Blocks of *C. elegans* were then placed on the NGM and the survival of worms was assayed. Results showed that the percentage survival of *C. elegans* decreased as the glucose concentration in the NGM agar increased, while survival rates of *C. elegans* with herb extracts were too low and inconsistent to show accurate readings. The percentage survival of *C. elegans* under ginseng was inconsistent with our original hypothesis, due to a few limitations, such as very few *C. elegans* being inside the transferred blocks of *C. elegans* and *C. elegans* being hidden under the opaque ginseng extract.

1. Introduction

Diabetes mellitus is a disorder in which the body does not produce enough or respond normally to insulin. When this happens, less of the glucose ingested can be stored as glycogen, hence the concentration of glucose in the blood rises. High-glucose diets are known to generate reactive oxygen species which can damage lipids, proteins and nucleic acids by causing oxidative stress (Alcántar-Fernández et al., 2018). There can be severe complications which arise, with life-threatening consequences of heart disease, stroke, kidney disease and nerve damage, the latter which can lead to serious, hard-to-treat infections (American Diabetes Association, 2011).

Diabetes mellitus is a worldwide major public health problem, and is becoming more prevalent in today's society, with 1 in 9 people in Singapore aged 18 to 69 having diabetes mellitus, and 1 in 3 people in Singapore having a lifetime risk of developing diabetes mellitus. Even with more technologically advanced solutions which are more efficient and cost-effective, people with diagnosed diabetes incur average medical expenditures of $16,752 per year, of which about $9,601 is attributed to diabetes. In addition with the fact that there is no cure for diabetes at all, even at type 1, this would result in a lifetime of medical debt. For the aforementioned reasons, a cheaper alternative such as herbs would be a suitable replacement solution for treating diabetes mellitus in the long term.

Some herbs have properties that are able to counteract diabetes mellitus by lowering the blood glucose level. Bitter melon (*Momordica charantia*) extract from the fruit, seeds, and leaves have been shown to contain several bioactive compounds that have hypoglycemic activity in both diabetic animals and humans. Saponins isolated from *M. charantia* showed significant insulin releasing activity. Hypoglycaemic agents that have been isolated from bitter melon are charantin, polypeptide-p and vicine (Joseph & Jini, et al. (2013) evaluated the antihyperglycemic and anti-obese effects of *Panax ginseng* berry extract and its major constituent, ginsenoside Re, in obese diabetic mice. The administration of *P. ginseng* berry improved systemic insulin sensitivity and glucose homeostasis, hence suggesting its clinical importance in the management of type 2 diabetes mellitus.

Caenorhabditis elegans is a suitable model organism to study glucose toxicity, in which high glucose conditions limit its lifespan by increasing the formation of reactive oxygen species. Because of its short life span and its simple insulin receptor system (daf-2), *C. elegans* can be used to study molecular targets affected by high glucose concentrations and how they can reduce its lifespan (Schlotterer et al., 2009). It has been established that a shortened lifespan in glucose-fed worms is in part due to the activation of the insulin/IGF-1 signaling (IIS) pathway (Alcántar-Fernández et al., 2018). Hence it is of interest to find out if herbs such as ginseng and bitter melon are able reverse the effects of glucose toxicity in *C. elegans* by increasing its life span.

2. Objectives and hypotheses

We hypothesised that increasing glucose concentration would result in increasing toxicity in *C. elegans*, reducing its survival, and that the administration of herbs could restore the survival of *C. elegans* exposed to glucose.

Our objectives were to investigate the effect of increasing glucose concentration on the survival of *C. elegans*, and whether herbs are able to reverse glucose toxicity in *C. elegans*.

3. Methods and Materials

Growth of bacterial preculture

Escherichia coli OP50 was inoculated into 10 ml of LB (Luria-Bertani) broth and grown overnight at 30°C in a shaking incubator. The absorbance of the preculture at 600 nm was measured using a UV-vis spectrophotometer and was standardised at 0.8.

Preparation of herb extracts

25 g of ginseng was blended separately in 100 ml of deionised water. The mixture was centrifuged at 7000 rpm for 10 min, the supernatant was collected and filter-sterilised.

The herbs were obtained from National Trades Union Congress FairPrice Xtra VivoCity.

Preparation of NGM (Nematode Growth Medium) agar

The composition of NGM (supplemented with glucose) was as follows: 0.9 g NaCl, 7.5 g agar, 0.75 g bacto peptone, and glucose (0, 100, 200 mM, 400 mM) in about 240 ml deionised water. After autoclaving, 0.3 ml cholesterol (5 mg/ml), 0.3 ml $MgSO_4$ (1 M), 0.3 ml $CaCl_2$ (1 M), 7.5 ml KH_2PO_4 solution pH 6.0 (1M) were added.

NGM agar was also supplemented with 60 ml of herb extract (test setup) or deionised water (control setup). After mixing well, the agar was poured into Petri dishes and allowed to solidify.

C. elegans survival and locomotion assay

0.05 ml of *E. coli* OP50 preculture was then added to NGM plates and grown for 24 h overnight at 30°C, as food source for *C. elegans*.

A block of agar containing *C. elegans* N2 was then placed on the centre of the NGM plate with varying concentrations of glucose and supplemented with herb extracts (test setup) or sterile water (control setup) and incubated at 25°C for another 2 days.

The percentage survival, speed of locomotion and number of body thrashes per minute of *C. elegans* were determined using the WormLab system (MBF Bioscience).

In summary, five replicates of each of the following setups were prepared for each assay:

C. elegans grown on 0 mM glucose without herb
C. elegans grown on 100 mM glucose without herb
C. elegans grown on 200 mM glucose without herb
C. elegans grown on 400 mM glucose without herb

C. elegans grown on 0 mM glucose with ginseng extract
C. elegans grown on 100 mM glucose with ginseng extract
C. elegans grown on 200 mM glucose with ginseng extract

C. elegans grown on 400 mM glucose with ginseng extract

4. Results and Discussion

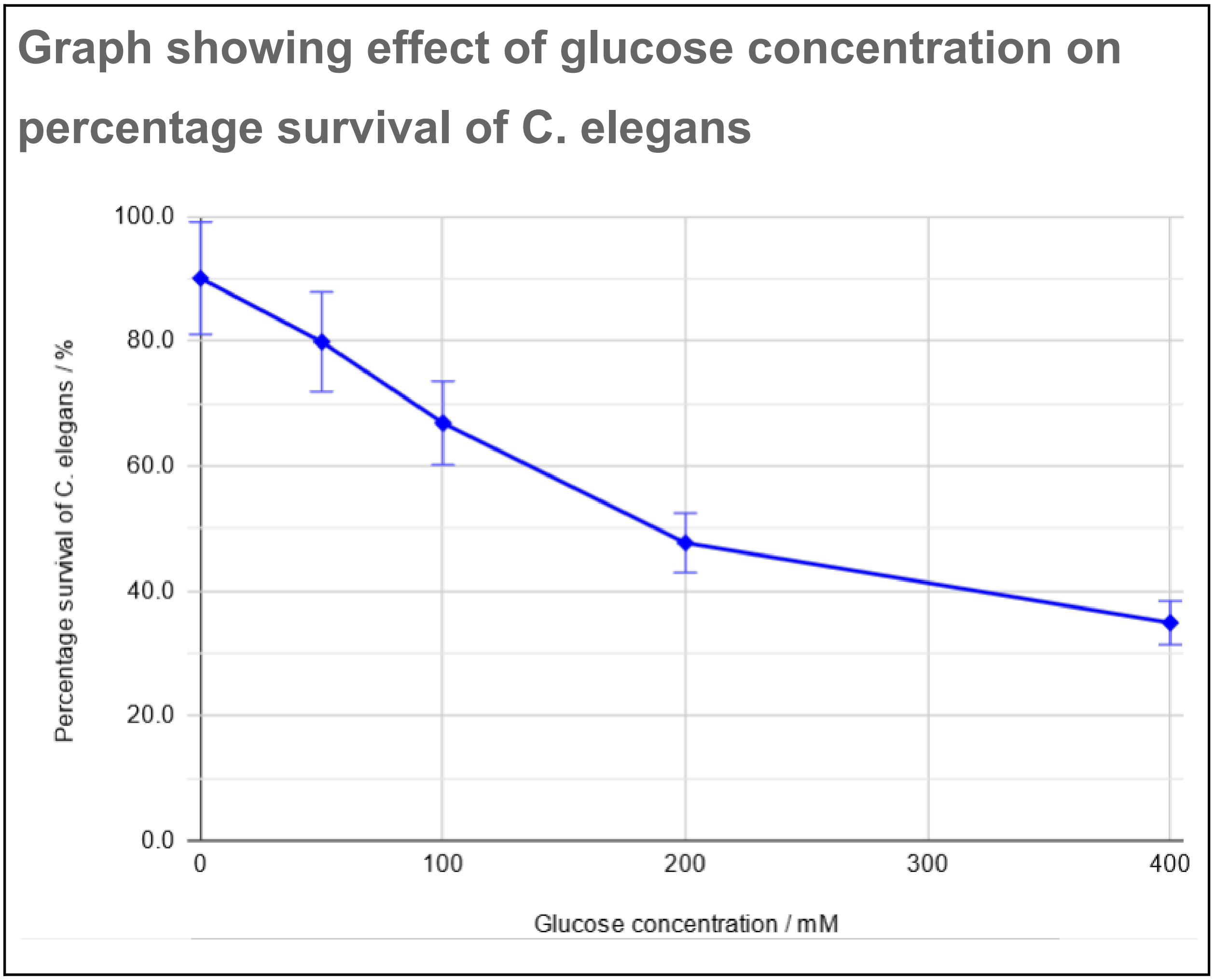

Fig. 1: Graph showing the effect of glucose concentration on percentage survival of *C. elegans*

As shown in Fig. 1, the percentage survival of *C. elegans* in Experiment 1 decreased from 90.1 to 34.9 percent, per petri dish, as the glucose concentration per petri dish increased from 0 to 400 mM. The highest percentage survival of *C. elegans* was recorded in the petri dish with 0 mM glucose concentration, while the lowest percentage of *C. elegans* were recorded in the petri dish with 400 mM glucose concentration.

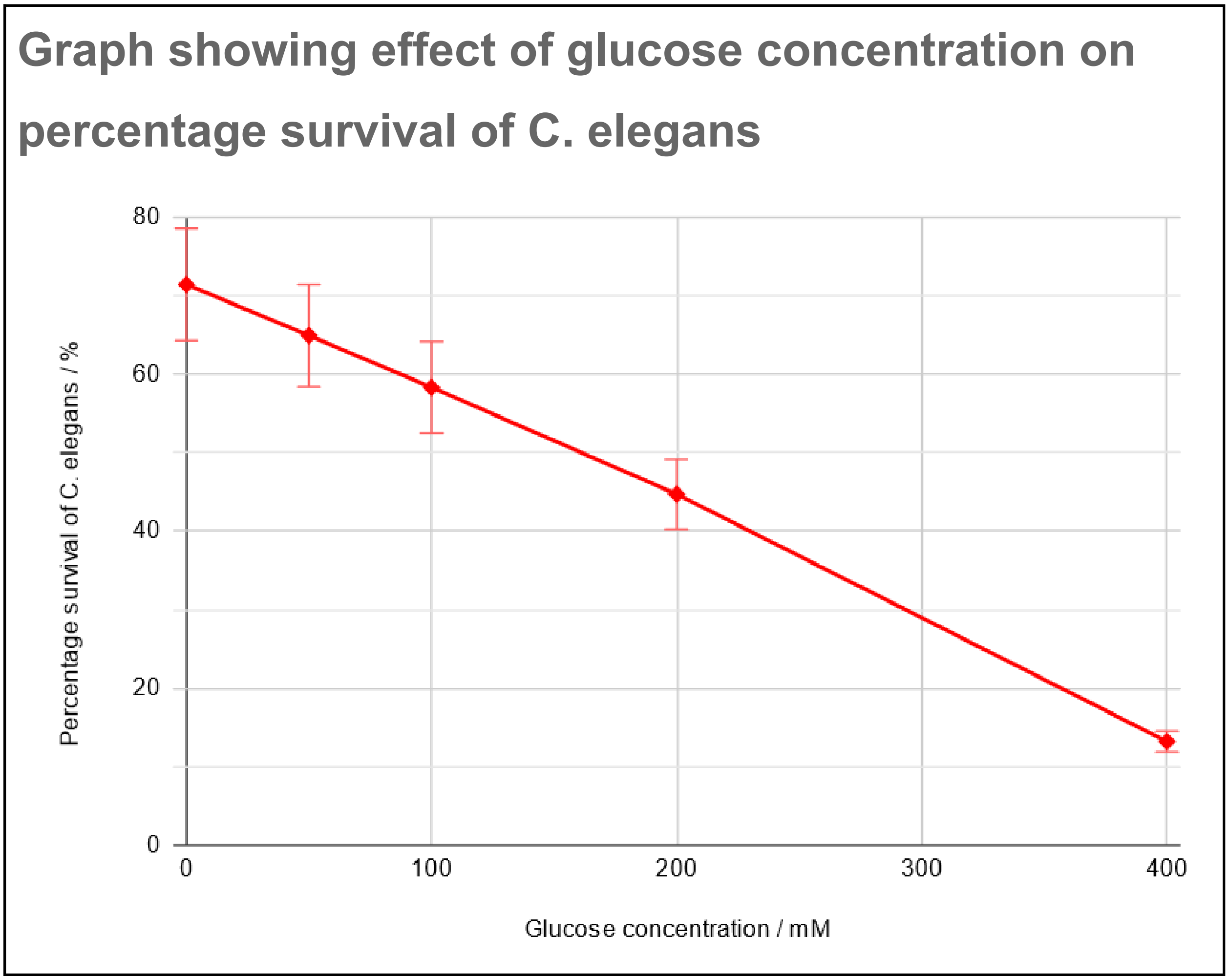

Fig. 2: Graph showing the effect of glucose concentration on percentage survival of *C. elegans*

As shown in Fig. 2, the percentage survival of *C. elegans* decreased from 71.4 to 13.2 percent, per petri dish, as the glucose concentration per petri dish increased from 0 to 400 mM. The highest percentage survival of *C. elegans* were recorded in the petri dish with 0 mM glucose concentration, while the lowest percentage survival of *C. elegans* were recorded in the petri dish with 400 mM glucose concentration.

Fig. 1 and Fig. 2 have similar trends, with a deviation not exceeding 25 percent at any given point in the graph. The graphs present a consistent decrease in the percentage survival of *C. elegans* directly proportional to the increase in glucose concentration.

The percentage survival of *C. elegans* under ginseng were inconsistent with our original hypothesis. This could be due to a few limitations. Firstly, there were originally very few *C. elegans* inside the transferred blocks of *C. elegans*. Secondly, *C. elegans* were hidden under the thick ginseng extract, so dead *C. elegans* were not very visible as it blended in with the colour of the ginseng. Live C*. elegans* were likewise hard to spot if they were juveniles. There were not enough *C. elegans* to accurately represent the results of the experiment, and more *C. elegans* had to be recorded to provide more accurate and comprehensive data on the percentage survival of *C. elegans*.

The reason why the increase in glucose concentration caused a decrease in percentage survival of the *C. elegans* was because of glucose toxicity. *C. elegans* cultured under high glucose conditions increased in vivo intracellular glucose concentrations, and generated more reactive oxygen species (ROS), carbonylated proteins and advanced glycation end product (AGE) modification of mitochondrial proteins in a daf-2 independent manner (Schlotterer, et al.).ROS and carbonylated protein accelerated aging of *C. elegans*, while increased levels of fluorescent AGEs indicated enhanced glycation in *C. elegans* under high glucose conditions (Ou, 2020).

5. Conclusion and Recommendations for future work

Increasing glucose concentration results in higher glucose toxicity in *C. elegans*, reducing their percentage survival. High amounts of glucose would result in certain death for *C. elegans*.

Ginseng results in a decrease in percentage survival of *C. elegans*, adversely affecting *C. elegans* which ingest it. This result did not agree with previous research studies on the positive impact of ginseng in reducing glucose toxicity.

C. elegans is a good model organism to study glucose toxicity in humans. Diabetes mellitus can be developed in *C. elegans*, with high glucose conditions increasing ROS formation and AGE modification of mitochondrial proteins in a daf-2 independent manner, limiting the lifespan of the *C. elegans*. The organism was hence also suitable for testing the effectiveness of herbs in reversing glucose toxicity. As such, high quantities of glucose are likewise not recommended for human consumption.

Different types of herbs and different (lower) concentrations of herbs could be used to further continue our experimental research. Further experiments on the individual parts of diabetes mellitus such as insulin production could be investigated. Negative effects of herbs on the survival rate of *C. elegans* could also be further investigated.

6. References / Bibliography

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