# Investigating the Effect of Quinine-Induced Autophagy Inhibition on the Efficacy of Curcumin Cancer Treatment

## Background and Purpose of the Research Area

Exposure to sunlight for prolonged periods can damage the skin, cause sunburn and increase the risk of certain types of cancer. The medium-wavelength UVB component of sunlight is one of the major risk factors for the development of skin cancer in humans. UVB light is known to induce oxidative stress by generating reactive oxygen species (ROS), which are responsible for oxidative damage to proteins, DNA, RNA and lipids (Perluigi et al., 2010).

Autophagy is a normal physiology process where dysfunctional or damaged cellular contents are degraded and recycled during cellular stress. Autophagy plays an important role in cancer because of its tumour inhibiting and tumour protecting functions. In the early stages of tumour development, autophagy helps healthy cells suppress tumour growth by maintaining cellular homeostasis and degrading tumour-promoting proteins. However, in the later stages, autophagy helps the cancer cells resist cytotoxic stress from anti-cancer drugs. Quinine, which is a quinoline-based antimalarial compound, is a known autophagy inhibitor (Golden et al., 2015).

The genome of the nematode *Caenorhabditis elegans* has a high similarity with the human genome, hence making it a good model organism for anti-cancer drug discovery and cancer research (Kobet et al., 2014; Kyriakakis, Markaki, & Tavernarakis, 2014). UV-B light (302 nm) is used as a convenient way to induce large amounts of DNA damage in *C. elegans* (Lans & Vermeulen, 2011). This may model early stage cancer because of DNA damage.

Curcumin, a polyphenolic compound from the turmeric root has been researched extensively for the treatment of cancer in animals because of its anticancer and antiproliferative properties. It can inhibit tumour initiation and tumour promotion with minimal side effects (Ravindran, Prasad, & Aggarwal, 2009; Vallianou, Evangelopoulos, Schizas, & Kazazis, 2015; Zhou et al., 2017). Goji berry, scientifically known as *Lycium barbarum*, has been used in Traditional Chinese Medicine for more than 2000 years. It has been studied as a potential anticancer agent in humans because of its antioxidant and antiproliferative properties (Zhou et al., 2014; Wawruszak, Czerwonka, Okła, & Rzeski, W., 2015).

Few studies have combined both curcumin and quinine in the treatment of cancer. This study aimed to investigate if inhibiting autophagy using quinine enhanced the anticancer effects of curcumin and goji berry. The effects of curcumin and goji berry added with various concentrations of quinine on UVB-treated *C. elegans* were studied. The objective of this study was to investigate the optimal concentration of quinine an autophagy inhibitor with curcumin and goji berry in restoring the maximum survival of UV-treated *C. elegans*.

**Hypotheses of the Research**

We hypothesise that curcumin and goji berry could protect *C. elegans* against UV light-induced DNA damage, restoring survival and locomotion of *C. elegans*. Greater concentration of quinine, an autophagy inhibitor, with curcumin or goji berry would lead to decreased protection of *C. elegans* against UV-induced oxidative stress.

**Experimental Procedures**

**Preparation of curcumin, goji berry and quinine solutions**

0.01 g of curcumin or 0.01 g of quinine was blended in 100 ml of water and filter sterilised with a microfilter. 10 g of goji berries were ground with a mortar and pestle and mixed with 100 ml of water. The mixture was centrifuged for 10 min at 7000 rpm and filter sterilised.

**DPPH test**

The DPPH test was conducted to determine the antioxidant activity of curcumin and goji berry. 1,1-diphenyl-2-picryl-hydrazil (DPPH) is a free radical which produces a purple solution when dissolved in methanol. When it is reduced by antioxidants, a change in colouration from purple to yellow is observed. The negative control consisted of 1.0 ml of DPPH, 1.9 ml of methanol and 0.1 ml of sterile water. In the test set-ups, curcumin and goji berry extracts replaced the sterile water. Five replicates were prepared. For the respective blanks for each set-up, methanol was added instead of DPPH solution. The initial absorbance was measured at 517 nm against the respective blanks and the mixtures were then left to stand in the darkness for 20 min, before the final absorbance readings were measured. The % radical scavenging activity is calculated as follows:

(Final absorbance of control – Final absorbance of test)/Final absorbance of control x 100%

**Growth of bacteria**

*Escherichia coli* OP50 was grown in 10 ml of LB broth overnight at 30°C in a shaking incubator. This served as food for the nematode *C. elegans*.

**Preparation of NGM medium for *C. elegans***

The composition of NGM was as follows: 0.9 g NaCl, 7.5 g agar, 0.75 g bacto peptone in 300 ml 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 potassium phosphate buffer pH 6.0 (1 M) were added.

**Treatment of *C. elegans* with curcumin, goji berry and quinine**

Curcumin, goji berry and quinine were dissolved in water and 0.5 ml of each was spread onto NGM plates. 0.05 ml of *E. coli* OP50 broth culture was added to the NGM plates and grown overnight at 30°C. A block of agar containing *C. elegans* N2 was placed at the centre of the NGM plate. The plate was incubated at 20°C for 2 days for the growth of *C. elegans*. After 2 days, *C. elegans* was collected with M9 buffer and centrifuged for 1 min at 2000 rpm.

### Inducing oxidative stress in *C. elegans*

A Petri dish containing the mixtures in the test and control setups was placed on the UV transilluminator. UV light of wavelength of 302 nm was shone on *C. elegans* for 2 min to induce DMA damage and oxidative stress. Five replicates were prepared. The percentage survival of *C. elegans* was determined. The software WormLab was used to determine the locomotion of *C. elegans* in all the setups. A summary of the setups are presented in Table 1.

**Table 1:** Summary of treatments

| | |
|---|---|
| **Test** | UV-treated *C. elegans* treated with curcumin/goji berry and quinine |
| **Control (1)** | UV-treated *C. elegans* treated with curcumin/goji berry only |
| **Control (2)** | UV-treated *C. elegans* without curcumin/goji berry |
| **Control (3)** | No UV-treated *C. elegans* |

## Results

### DPPH antioxidant test

Goji berry was shown to possess antioxidant activity through the DPPH test with a radical scavenging activity (RSA) of 38.6%. The final absorbance of the test setup with goji berry was significantly lower than that of the control (Mann-Whitney U test p value of 0.012). However, there was no significant difference in the final absorbance between the control and the test setup with curcumin (Mann-Whitney U test p value of 0.144), indicating that no antioxidant activity was detected via the DPPH test for curcumin. This is shown in Fig. 1.

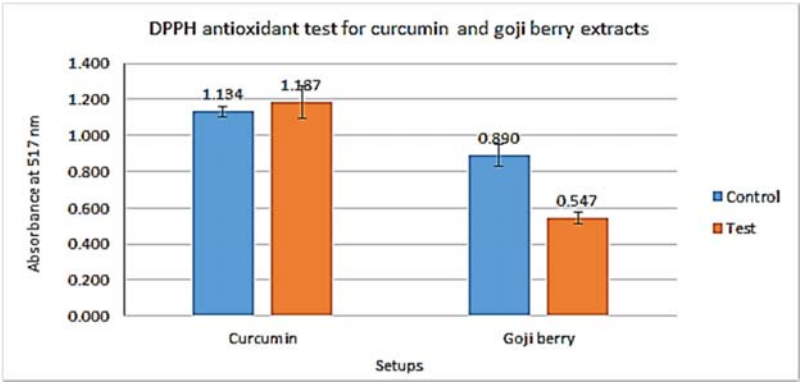


**Fig. 1:** DPPH antioxidant test for curcumin and goji berry extract.

### Effect of curcumin and quinine on UV-treated *C. elegans*

UV-treated *C. elegans* added with curcumin resulted in a significant increase in the percentage survival as compared to those without addition of curcumin (Mann-Whitney U test p value of 0.012 for setups UV vs UV+C.) There was a significant difference between the percentage survival of *C. elegans* treated with curcumin and curcumin with quinine (Kruskal Wallis test p value of 0.000). As the concentration of quinine increased, the percentage survival

of UV-treated *C. elegans* increased, although it was lower than treatment with curcumin alone. The setup with the highest percentage of UV-treated *C. elegans* that survived with curcumin and quinine treatment occurred when the quinine concentration was 80 µg/ml. However, there was no significant difference between the percentage survival of UV-treated *C. elegans* with curcumin alone and this particular setup (Kruskal Wallis test p value of 0.117 for setups UV+C vs UV+C+Q80). C represents curcumin only and C+Q80 represents the setup with curcumin and 80 µg/ml quinine. Results are shown in Fig. 2.

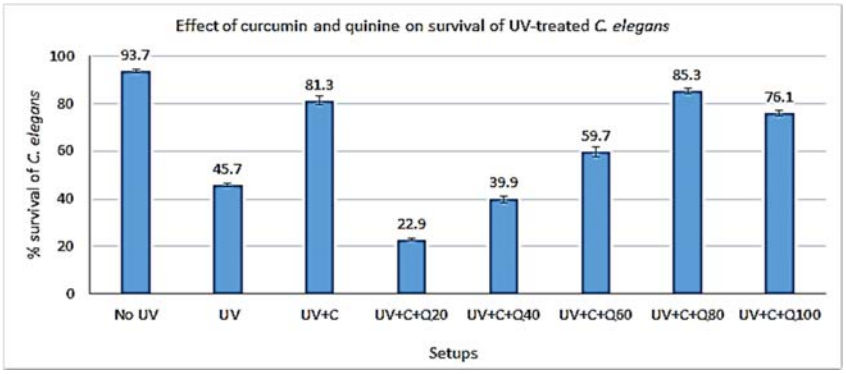


**Fig. 2:** Effect of curcumin and quinine on survival of UV-treated *C. elegans.*

UV-treated *C. elegans* added with curcumin resulted in a significantly higher speed of movement as compared to those without curcumin (Mann-Whitney U test p value of 0.000 for setups UV vs UV+C.) There was a significant difference between the speed of movement of *C. elegans* treated with curcumin and curcumin with quinine (Kruskal Wallis test p value of 0.000). As the concentration of quinine increased, the speed of movement of UV-treated *C. elegans* increased, although it was still lower than treatment with curcumin alone. The setup with the highest speed of movement of curcumin and quinine treatment occurred when the quinine concentration was at 80 µg/ml. However, there was no significant difference between the speed of movement of UV-treated *C. elegans* with curcumin alone and this setup (Kruskal Wallis test p value of 0.321 for setups UV+C vs UV+C+Q80). This is shown in Fig. 3. A similar trend for the bending angle of *C. elegans* was observed (Fig. 4).

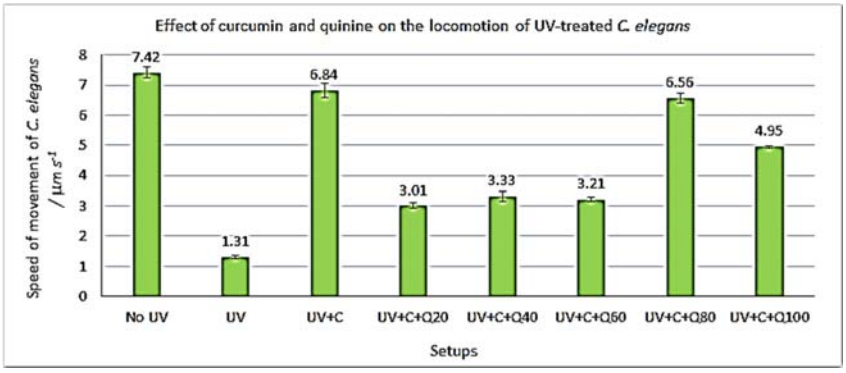


**Fig. 3:** Effect of curcumin and quinine on the locomotion of UV-treated *C. elegans.*

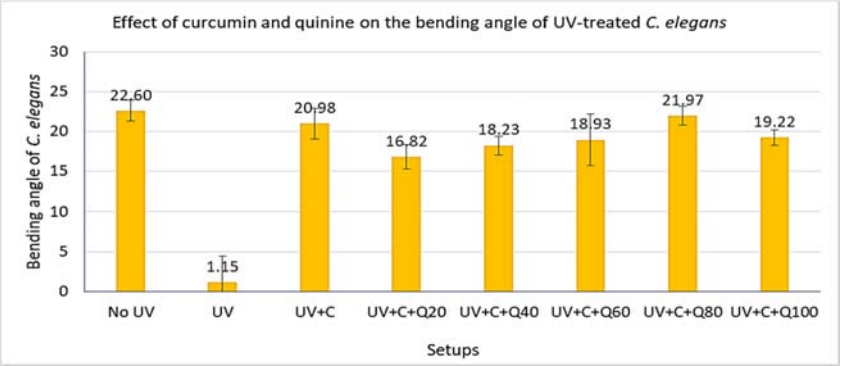


**Fig. 4:** Effect of curcumin and quinine on the bending angle of UV-treated *C. elegans.*

**Effect of goji berry and quinine on UV-treated *C. elegans***

UV-treated *C. elegans* added with goji berry extract resulted in a significant increase in percentage survival as compared to those not treated with goji berry (Mann-Whitney U test p value of 0.012 for setups UV vs UV+G). There was a significant difference between the percentage survival of *C. elegans* treated with goji berry alone and goji berry with quinine (Kruskal Wallis test p value of 0.001). As the concentration of quinine increased, the percentage of UV-treated *C. elegans* that survived increased. The optimal treatment with the highest percentage survival of UV-treated *C. elegans* was with goji berry and quinine at concentrations of 60 µg/ml, i.e. setup UV+G+Q60. The results are shown in Fig. 5.

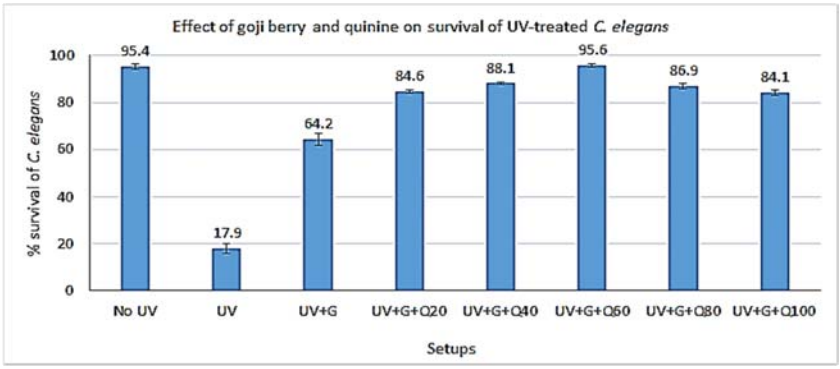


**Fig. 5:** Effect of goji berry and quinine on survival of UV-treated *C. elegans.*

The locomotion test showed the same trend as the survival of *C. elegans*. UV-treated *C. elegans* fed with goji berry resulted in a significantly higher speed of movement as compared to those without goji berry treatment (Mann-Whitney U test p value of 0.000 for setups UV vs UV+G). There was a significant difference between the speed of movement of *C. elegans* treated with goji berry and goji berry with quinine (Kruskal Wallis test p value of 0.000). As the concentration of quinine increased, the speed of movement of UV-treated *C. elegans* increased. The optimal treatment with the highest speed of movement was with goji berry and quinine at concentrations of 60 µg/ml, i.e. setup UV+G+Q60. Results are shown in Fig. 6. A similar trend was also observed for the bending angle of *C. elegans* (Fig. 7).

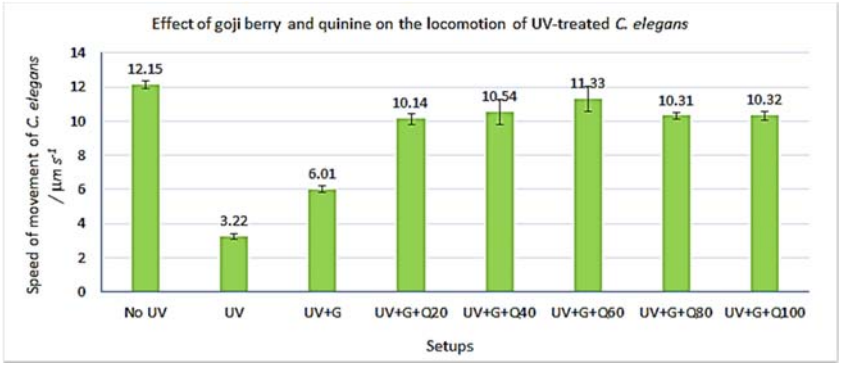


**Fig. 6:** Effect of goji berry and quinine on the locomotion of UV-treated *C. elegans.*

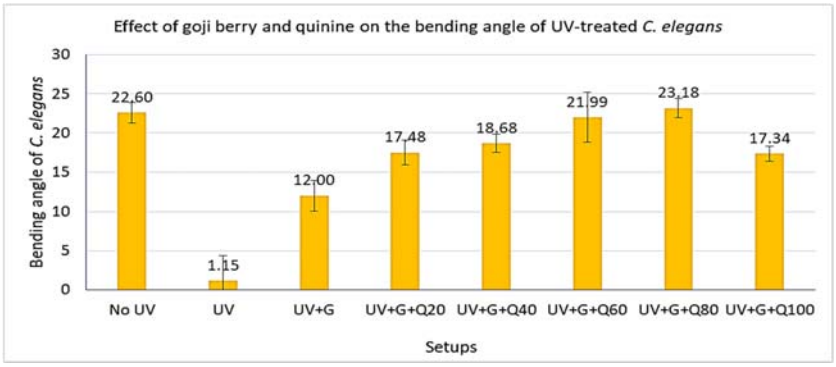


**Fig. 7:** Effect of goji berry and quinine on the bending angle of UV-treated *C. elegans.*

(a) (b)

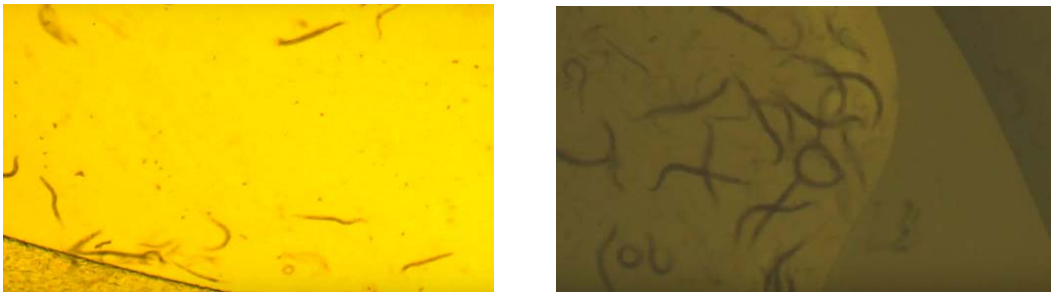

**Fig. 8:** Appearance of UV-treated *C. elegans* (a) in the control setup without curcumin or goji berry extract, and (b) with goji berry extract. There were lower survival, locomotion and bending angle of worms in the control setup.

**Conclusions and Discussion**

The results of this study showed that the optimal concentrations of quinine, an autophagy inhibitor, that restored the highest survival of *C. elegans* were found to be 80 µg/ml and 60 µg/ml when used in combination with curcumin and goji berry, respectively. In the initial stages of DNA damage, quinine should not be added to curcumin as this reduced the efficacy of *C. elegans* treated with curcumin to remove damaged organelles. In contrast, the addition of quinine with goji berry increased the survival of UV-treated *C. elegans*, suggesting an initiation of DNA repair.

Autophagy plays both a pro-survival and pro-death role in cells. When subjected to stresses like starvation, autophagy removes damaged organelles or protein aggregates and recycles metabolic substrates to maintain energy homeostasis, playing a pro-survival role. However, excessive levels of autophagy promote autophagic cell death and plays a pro-death role (Kang, You & Avery, 2007; Dalby, Tekedereli, Lopez-Berestein, & Ozpolat, 2010).

The results of this study have shown that quinine, a known autophagy inhibitor, has different effects when added to curcumin and goji berry. Curcumin is a naturally occurring autophagy modulator that can remove dysfunctional organelles and provide an energy source or essential nutrients to maintain cellular activity (Ravindran et al., 2009; Shakeri, Cicero, Panahi, Mohajeri & Sahebkar, 2018). Addition of quinine seemed to interfere with the pro-death role of curcumin to remove damaged organelles and made it less effective in promoting the survival of *C. elegans*. Goji berry has been reported to have antioxidant and anticancer properties (Zhou, et al., 2014; Wawruszak et al., 2015). Addition of quinine to goji berry treatment hence increased the survival rate of UV-treated *C. elegans* as it enhanced its pro-survival role. Therefore, different levels of autophagy when used in combination with curcumin or goji berry either increased or decreased the survival of *C. elegans*.

Hence the results of this study can be applied in anticancer therapy. The optimal level of an autophagy inhibitor, when supplemented with anticancer agents, can be used to enhance treatment of cancer and can potentially be incorporated in chemotherapy drugs against various types of cancer.

A limitation of this study is the lack of a fluorescence microscopy analysis that is needed to establish the level of autophagy under the various treatments. Despite the fact that *C. elegans* is a good model organism to show human diseases, it is not known if the same trend observed in this study can be extended to human cancer cell lines.

As future work, the level of autophagy can be monitored using fluorescence microscopy so that the effect of quinine can be better ascertained. A combination of curcumin and goji berry can also be tested to determine if any synergistic effects in restoring survival of UV-exposed *C. elegans* can be observed.

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