Investigating the effects of traditional herbs and IGF-I on the production of collagen in animal cells

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1. Introduction

1.1 Abstract

Tendinosis occurs when type-I collagen that composes the tendon degenerates through repetitive motion and ageing. As studies have shown that daily use of *Cissus quadrangularis* (*C.quad*) increases collagen production in the body, *C.quad* has great potential to be an active ingredient for an alternative cure for tendinosis. In addition, *Rhizoma homalomenae* (*R.homa*) and *Erycibe obtusisifolia* (*E.obtusi*) are common herbs used in Traditional Chinese Medicine to promote tendon health and could be used in combination with *C.quad* to treat tendinosis.

There are 3 phases in our project: Phase 1- Preparation of the herbal extracts, Phase 2- Screening the herbal extracts against the animal fibroblast cells and lastly, Phase 3 – Determining the amount of collagen produced. In Phase 1, the different herbal extracts were prepared and were screened in Phase 2 before being analysed in Phase 3 to see if there was any increase in collagen production in the animal fibroblast cells. Our findings showed that all 3 herbs *C.quad*, *R.homa* and *E.obtusi* increased collagen production by 43%, 27% and 52.5% ,compared to control, respectively.

Findings from our project can provide information to find a new natural herbal treatment for tendinosis which will have fewer side effects. Results of this project will serve to indicate the suitability of *C.quad, R.homa* and *E.obtusi* for use as an alternative treatment for tendinosis.

1.2 Background Information

Fibroblast cells are embedded in the extracellular matrix of tendons, which is composed of collagen, elastin, and proteoglycans (Robinson, 2002). Collagen is the main component of tendons, with type I collagen being the most abundant (Robinson, 2002).

Tendon disorders are a major problem in competitive and recreational sports, and one of the most common disorders is tendinosis, which occurs when collagen that composes the tendons degenerate (Clancy, 1990). Tendons may also degenerate due to repetitive motion and ageing (Summers, 2003). During the degeneration process, collagen breaks down and the body will respond by increasing collagen production (Erisson, 2004). Use of non-sterodial anti-

inflammatory drugs combined with rest and gradual return to exercise is a common therapy, although there is evidence to suggest that tendinosis is not an inflammatory disorder and that anti-inflammatory drugs are not an effective treatment (Bonar, 2002). Current treatments for tendinosis still involve the use of anti-inflammatory drugs and have proven to be ineffective, taking months to heal for such injuries (Kan *et al*, 2002).

This project aims to study the ability of the herbal extract of *C.quad*, *R.homa* and *E.obtusi* to enhance the production of collagen in animal cells. We hope that our findings will pave the way for the discovery of an alternative treatment for tendon injuries.

Cissus quadrangularis, an ancient herb found in India, is said to cause an increase in collagen production and aid the healing of joint ailments (Jainu and Mohan, 2008). It is the active ingredient found in medication used to relieve joint pain and increases the rate of recovery from tendon injuries (Cortes *et al*, 2004).

Insulin-like growth factor 1 (IGF-I) is a growth hormone that has been shown to be an in vitro stimulant of type I collagen synthesis, along with insulin and ascorbic acid (Ivarsson, 1998). In vitro treatment of tenocytes with IGF-1 resulted in an increased production of type I collagen (Tang *et al*, 2005). Studies have also shown that production of type I collagen in human fibroblast cells are increased by IGF-1 (Jonsson *et al*, 1980; Borg *et al*, 1998).

Homalomena rhizome is a herb used in Traditional Chinese Medicine to treat pain, numbness, and tightness of tendons (John and Tina , 2003). *Erycibe obtusifolia* is one of the active ingredients found in a common TCM herbal supplement known as Vine Essence Teapill that supports the health and well-being of bones, muscles, ligaments and tendons.

Recent research have shown that healing is dependent on early granulation of defects, maximizing collagen type I production, organization, elasticity, and minimizing scar tissue formation (Sutter, 2007). There are several cell-based treatments of tendon injuries that are currently being investigated, such as bone marrow aspirate, platelet-rich plasma, and adult mesenchymal tissue-derived stem cells.
The research angle adopted by this project is different from the other studies, in that instead of using stem cells to re-grow tendons, *Drosophilia* epithelial cells will be treated with IGF-1,

C.quad, R.homa and *E.obtusi* extractsto see if production of type I collagen can be enhanced. Data obtained will contribute to the limited pool of research of effects of the herbs on collagen production.

1.3 Hypothesis

Cissus quadrangularis, Rhizoma homalomenae and *Erycibe obtusisifolia* increase production of collagen synthesis in *Drosophilia* epithelial cells

2. Method

A) Preparation of extracts

Dried powder of *C.quad* was mixed with deionized water at concentration 4mg/ml. The *C.quad* extract was then centrifuged and sterile filtered using a 0.22µm sterile filter. 50µg of IGF-1 powder was mixed with 100ml of deionized water before being topped with 980µl of deionized water. 4g of *R.homa* and *E.obtusi* herbs were each soaked in 150ml of water and boiled for 30 minutes. The residue was filtered out and the extracts were sterile filtered using a 0.22µm sterile filter.

B) Treatment of cells

Drosophilia epithelial cells were cultured in an incubator at 22°C in 5ml of ATCC complete growth medium for 5 days. After 5 days, a hemocytometer was used to determine the cell density, and only flasks with cell density of 1.00 $x10^6$ per ml were used. For the experimental setup, *C.quad* extract was added into the cell cultures in concentrations (v/v) of 0.02%, 0.04%, 0.06%, 0.08% and 0.10%. *R.homa* and *E.obtusi* extracts were each added into the cell cultures in concentrations (v/v) of 0.01%, 0.02%, 0.03%, 0.04%, 0.05% and 0.06%. 2 other controls were set-up: for the positive control, IGF-1 extract was added to the cell culture in concentrations (v/v) of 0.004%, 0.012%, 0.02%, and the normal control comprised untreated cell culture in the ATCC complete growth medium only. The optimum concentrations for IGF-1 and *C.quad* were determined before the combination treatments were done and cell cultures were incubated at 22°C for 3 days before the Sircol Collagen Assay test was carried out

C) Sircol™ Collagen Assay

Collagen produced by the cells was quantified using *Sircol* Soluble Collagen Assay. The standard curve of collagen was first plotted. The cell suspensions were centrifuged at 4500RPM for a period of 10 minutes. 50µl of the medium was placed in microfuge tubes. 1ml of Sircol Dye reagent was added to each microfuge tube and the contents were mixed by inverting and shaking the tubes for 30 minutes. The microfuge tubes were then spun at 10,000RPM for a period of 10 minutes. The unbound dye solution was then removed by carefully inverting and draining the tubes before 1 ml of the alkali reagent was added to each microfuge tube. The contents were mixed using a vortex to allow the bound dye to dissolve before a spectrophotometer was used to read the absorbance of the sample at a wavelength of 540nm. The collagen content of the test samples will then be calculated from the collagen standard graph that has been plotted.

3. Results and Discussions

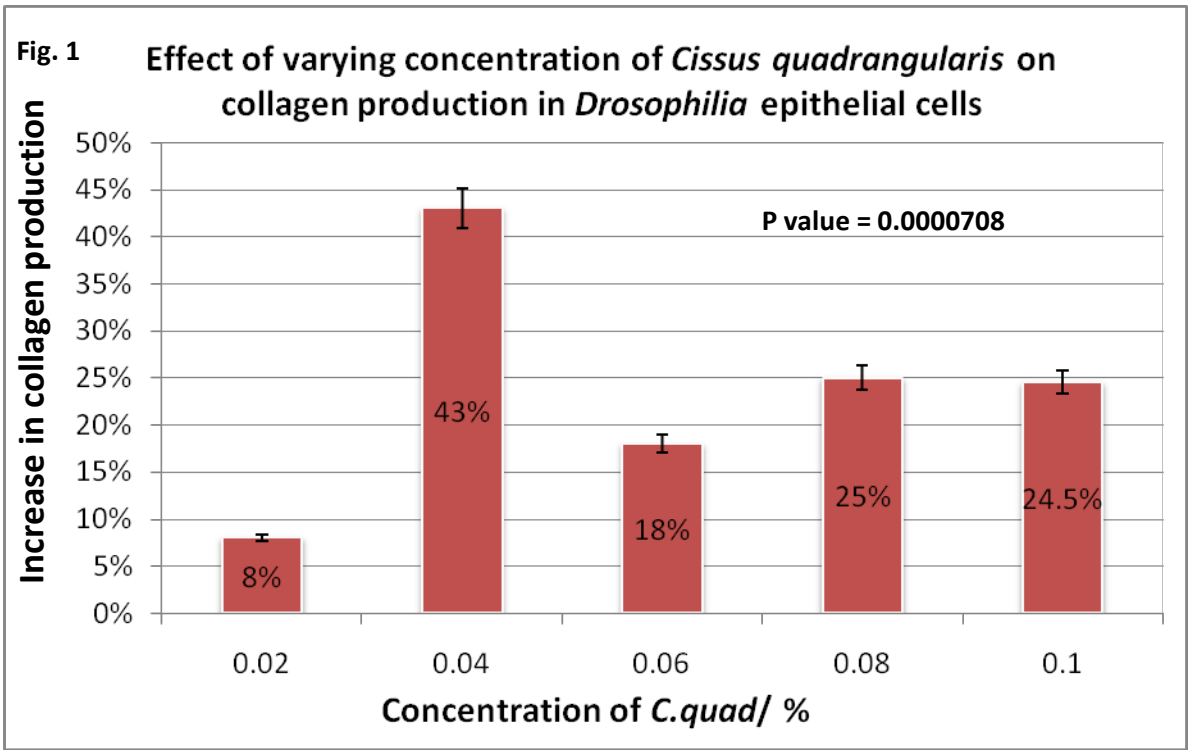


Fig. 1 shows effect of *C.quad* extract on collagen production. It was observed that at *C.quad* concentration of 0.04% (v/v), collagen production was stimulated to the greatest extent, with a 43% increase.

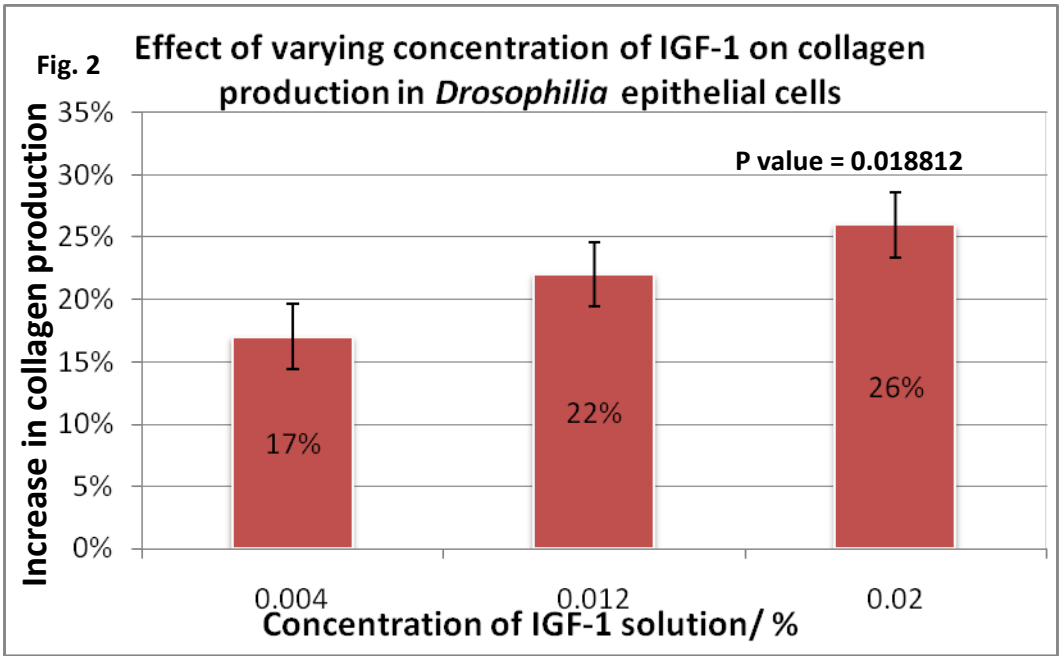
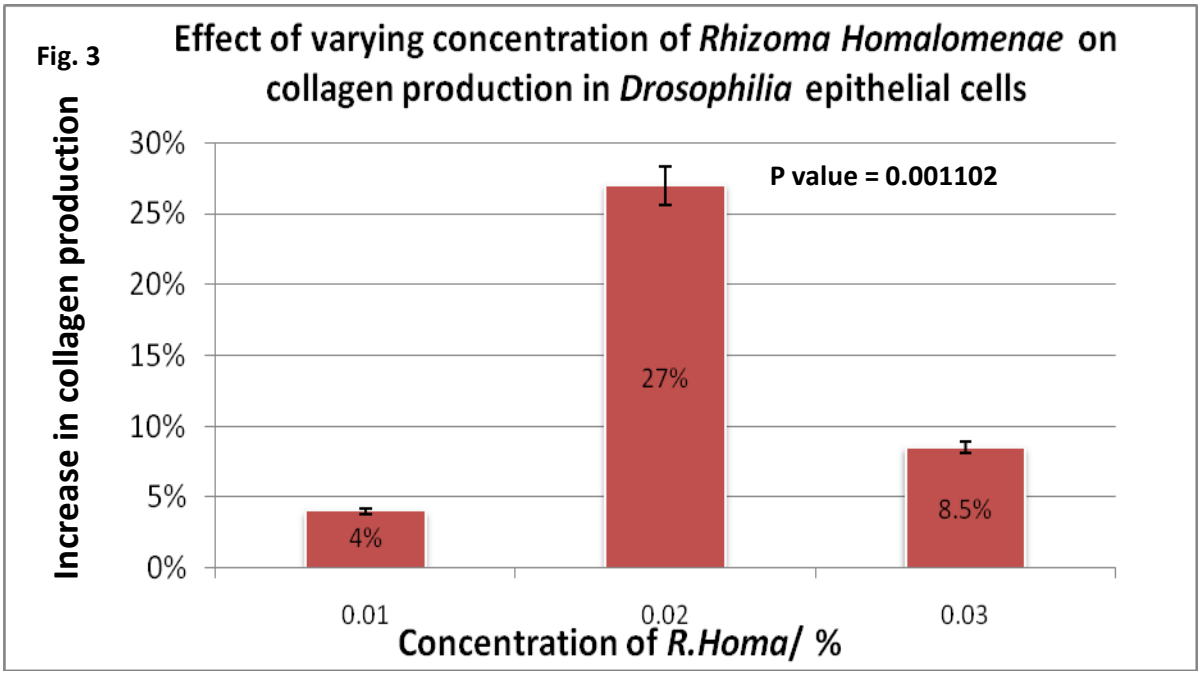


Fig. 2 shows the effect of IGF-1 extract on collagen production. At IGF-Iconcentration of 0.02% (v/v), collagen production was stimulated to the greatest extent, with a 26% increase.



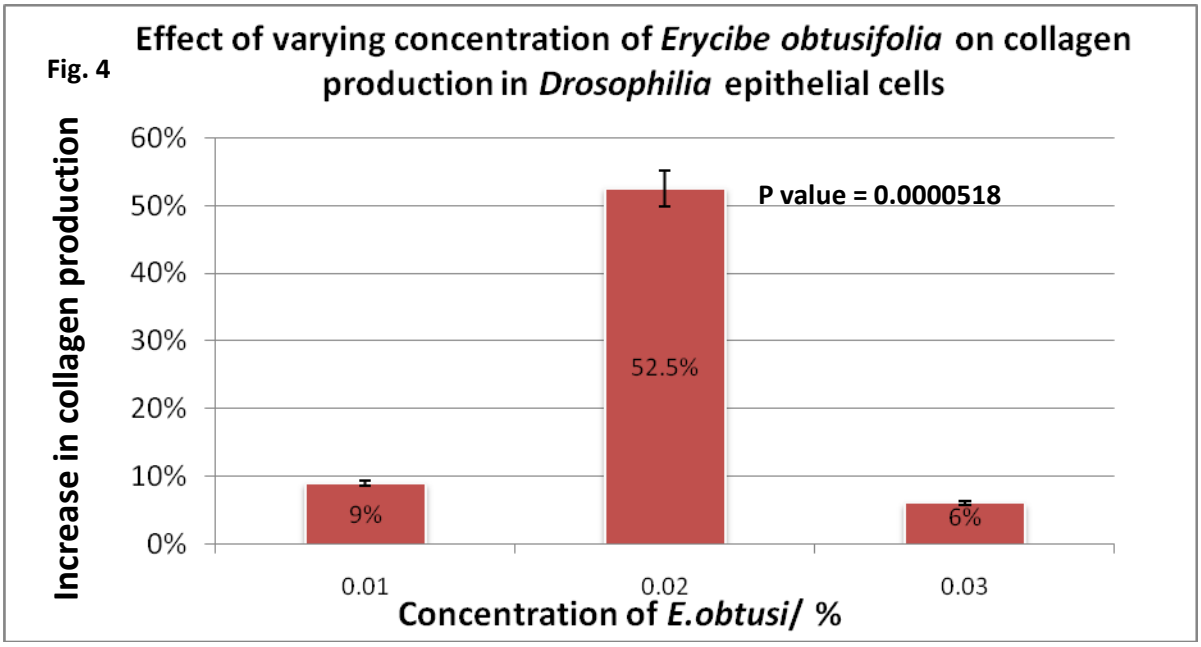
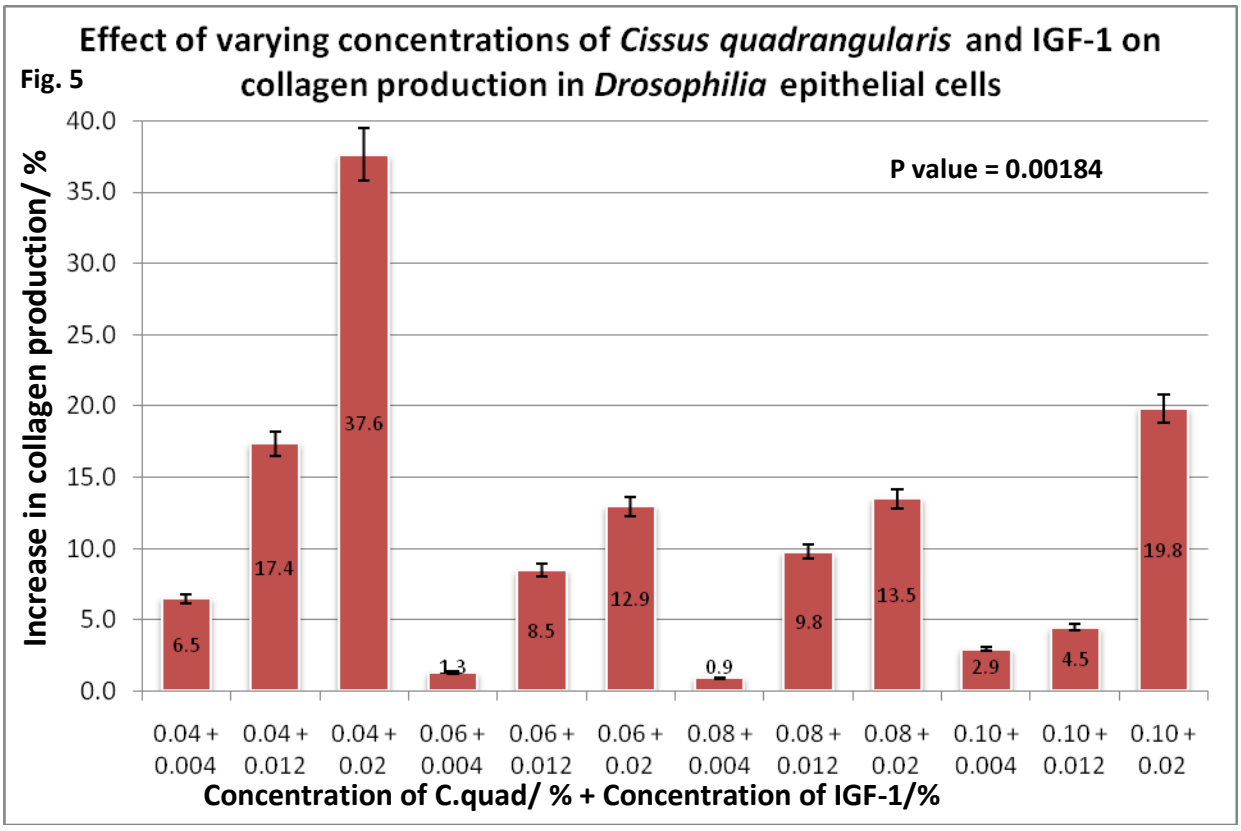


Fig. 3 and 4 shows the effects of *R.homa* and *E.obtusi* on collagen production respectively. It was observed that both *R.homa* and *E.obtusi* concentration of 0.02% (v/v), collagen production was stimulated to the greatest extent, with a 27% and 52.5% increase respectively.



The cells were also treated with a combination of *C.quad* and IGF-I. Fig. 5 shows the effect of this combination treatment on collagen production. For the combination treatments, it was observed that at *C.quad* concentration of 0.04% (v/v) and IGF-I concentration of 0.02% (v/v), collagen production was stimulated to the greatest extent, with a 37.6% increase.

Our findings showed that the amount of collagen produced, as a result of treatment with the different herbal extracts, was significantly greater than the amount of collagen produced in control trials (absence of herbal extracts). Hence our hypothesis was supported because all 3 herbal extracts were observed to increase collagen production by the *Drosophilia* epithelial cells. Furthermore, we found out that the stimulatory effect of a combination of *C.quad* and IGF-1 on collagen production is less than the effect of *C.quad* extract at concentration of 0.04%.

Application of *C.quad*, *R.homa* and *E.obtsui* could be used as a natural herbal treatment for tendinosis. They have the potential to be administered in the forms of injections or oral pills to treat tendinosis in the future.

3.1 Future work

Our project can be extended by carrying out the following:

1. Identify the active compounds in *C.quad*, *R.homa* and *E.obtusi* that are responsible for the effects observed.
2. Investigate the effects of *C.quad*, *R.homa* and *E.obtusi* on cell growth.
3. Screen other herbal extracts to determine their effectiveness to promote collagen production in these cells.

4. References

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