CALIFORNIA STATE SCIENCE FAIR
2003 PROJECT SUMMARY

Name(s)

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Project Number

J0404

Project Title

Does Red Clover Contain an Effective Phytoestrogen? Do Extracts of Red Clover Bind to the Estrogen Receptor?

Abstract

Objectives/Goals

The purpose of the experiment is to test how effectively red clover binds to the estrogen receptor (ER), and to see if the fresh plant extracts of red clover flowers and leaves bind to the ER more effectively than over-the-counter supplements. The hypotheses are that extracts of red clover will bind to the ER (as effectively as 17 beta-estradiol, a naturally active form of estrogen), and that the fresh plant extracts will bind more effectively than extracts from over-the-counter supplements.

Methods/Materials

MCF-7 cells were first transfected with ER plasmids and then test solutions (different concentrations of red clover from different sources) were mixed with the MCF-7 cells in 24-well plates and allowed to incubate for 48 hours to induce gene expression. Then the liquid media was aspirated (sucked out), and the cells remaining at the bottom were lysed with a bleach to get access to the DNA. The samples were then transferred to 240 Eppendorf tubes. Half the samples were treated with beta-galactosidase (an internal control) and the other half were treated with luciferin. The luciferase assay involves a reporter gene coupled to an estrogen response element so that light is produced by the presence of bound estrogen. So, the amount of ER binding was measured by the amount of light produced as measured by a luminometer.

Results

The results indicate that extracts of red clover from various sources do bind to the ER. The greatest luciferase activity was observed for the leaves of red clover. The flowers of red clover did not show as much activity as the leaves. Over-the-counter sources also showed luciferase activity but less than the leaves of red clover.

Conclusions/Discussion

This experiment demonstrated that extracts of red clover from various sources bind the ER. The leaves of red clover appeared to be the most effective. This is generally consistent with the hypotheses stated above. The results suggest that red clover may contain an effective phytoestrogen and further studies are warranted.

Summary Statement

This study addressed the question of whether red clover may contain an effective phytoestrogen by testing whether extracts of red clover bind to the estrogen receptor.

Help Received

This study was conducted at the UCLA Center for Human Nutrition. Dr. David Heber is Director of the Center and approved the project. Emily Besselink served as our mentor and helped us learn how to conduct all of the technical aspects of the experiment.