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## Effects of *Cimicifuga racemosa* on mammary gland development in mice

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*Cimicifuga racemosa*, also known as black cohosh is an herbal supplement that can alleviate the symptoms of menopause and other menses related ailments such as hot flashes, amenorrhea and inflammation of the uterus or ovaries. The mechanisms behind the effectiveness of black cohosh are not known because contradicting studies suggest that black cohosh has an estrogenic effect, where as other studies found that black cohosh has little or no estrogenic effect. Estrogen is a key component in the development of breast cancer. It is known that longer lifetime exposure to estrogen increases the risk of developing breast cancer. There are many cells or groups of cells in the body that are estrogen sensitive. In breast cancer, we are concerned with the group of cells in the mammary gland known as terminal end buds. Terminal end buds represent the structures where elongation and branching of the ducts occur and also where estrogen-stimulated cell division occurs. There is some evidence that links the number of terminal end buds to the risk of cancer because of the high proliferation rate of the cells at the end bud and its dependency of estrogen. Hence, this area is a prime target for cancer development. Because black cohosh can alleviate many estrogen related ailments we hypothesize that dietary black cohosh fed to pre-pubertal female mice would slow the mammary gland development, resulting in fewer terminal end buds in the black cohosh group compared to a control diet group. To determine if dietary black cohosh would alter development of the mammary glands, we performed the following experiment. Thirty-two female, C57blackJ mice were obtained from the MU colony at 3-4 weeks of age. The mice were randomly assigned to either a control diet or the same diet containing 0.04 % black cohosh. The black cohosh was obtained from Dr. Rachel Ruhlen, University of Illinois and was verified for authenticity. Mice were weighed and fed daily for 25 days. At completion of the dietary period, the mice were euthanized and the number four (4) mammary glands were excised from both sides. The mammary glands were fixed in formalin overnight and then were placed in a series of diluted ethanol solutions (70%, 50%, and 30%) for fifteen (15) minutes each. They were then placed in a 1% methylene blue solution for fifteen (15) minutes and stepped back in a series of ethanol solutions (70%, 90%, 95%, 100%) for fifteen (15) minutes each and rinsed with distilled water. For each whole mount two (2) microscope fields were selected and the number of terminal end buds were counted. In addition the size of the terminal end bud was ranked according to a scale from one to five (1 - 5) with one (1) being very small to five (5) being very large. The reader of the slides was blinded as to treatment. Each slide was quantified twice and the means compared using a T-test.