

## ANTICANCER EFFECTS OF LEECH EXTRACT ON BREAST CANCER CELLS

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**Introduction.** Throughout the history of humanity, leech therapy appeared as a procedure in medicine all over the world. The very first use of leech in human treatment was mentioned in Egypt 3500 years ago. In 2004, Food and Drug Administration approved leeches as medical devices and, nowadays, leeches are used in treatment of diseases, such as hypertension, varicose ulcer, thrombophlebitis and musculoskeletal diseases. In this experiment we are trying to see whether the leech saliva extract (LSE) has anti-cancer effects on MCF-7 breast cancer cells. Several studies were conducted previously that have shown positive effects of LSE on cancer cells. Warfarin and heparin from the extract are found to have antimetastatic effect. Experiments on lung tumor cells also indicated inhibitory effect of LSE.

**Methods.** LSE was collected from the starved leeches using 0.011 M arginine in normal saline as a phagostimulatory solution. Human breast cancer cell line MCF-7 was obtained from the American Type Cell Collection ATCC and cells were cultured with LSE for 3 days. The effect on cell division was checked using proliferation and viability assay. Soft agar assay was also conducted to show the ability of transformed cells grow independently of a solid surface, a hallmark of carcinogenesis. Following the incubation period of 28 days, the formed colonies were analyzed morphologically using stain.

**Results.** The assays demonstrated the tumor suppressive dose-dependent effects of LSE on MCF-7 cells. Proliferation assay showed much less number of MCF-7 cells after 3 days on incubation with LSE compared with nontreated cells. It was found that a concentration of 40 ug/ml resulted in about 35% cell growth inhibition and a concentration of about 8 ug/ml was able to induce a considerable percentage inhibition of approximately 15%. Cell staining demonstrated a considerable change in cytoskeleton structure of treated cells. The difference in size and quantity of colonies formed after LSE addition was also detected.

**Conclusion.** There are not many studies and experiments made on the effect of LSE on cancer cells. Primarily, the leech saliva is known for its anticoagulant and antimicrobial activity. However, the crude LSE contain a wide variety of peptides and proteins, which biological activities are still not fully studied. Because of the limited number of studies made on this topic, this project could contribute to the experimental analysis of the relatively new method of treating cancer.