**Vernonia amygdalina: A NOVEL BOTANICAL AGENT FOR THE TREATMENT OF BREAST CANCER**

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**Abstract**: Breast cancer (BC) is the leading cause of death of women between 40 and 55 years of age and is the second overall cause of death of women. Fortunately, the mortality rate from BC has decreased in recent years due to an increased emphasis on early detection and more effective treatments. Despite early detection and conventional methods of treatment, about 7% of women diagnosed with BC still die every year. Therefore, novel therapeutic agents are needed to improve the clinical outcome of this disease. The development of new drugs from natural products is considered important. Previous studies from our laboratory show that a novel natural product, extracts of *Vernonia amygdalina* (VA) leaf exerts DNA-damaging anticancer activities against BC. Therefore, the central goal of this research was to determine the therapeutic mechanisms of VA leaf extracts in breast cancer cells. To achieve this goal, cell viability, live and death cells were determined by the means of the MTT assay, trypan blue test, and propidium iodine assay, respectively. Cell apoptosis was measured by flow cytometry analysis of phosphatidylserine externalization (Annexin V assay) and caspase 3 activity, and by DNA laddering assay. Data obtained from the MTT assay indicated that VA significantly reduced the viability of MCF-7 cells a dose-dependent response. On one hand, the Trypan blue dye exclusion test demonstrated the integrity of the membrane of untreated cells in culture. On the other hand, the trypan blue dye exclusion test demonstrated a loss of viability in VA-treated cells due to membrane damage. The result of the propidium iodine demonstrated a significant (p<0.05) increase of necrotic cell death in VA-treated cells, indicative of membrane rupture by VA. Flow cytometry data showed a strong dose-response relationship between VA exposure and Annexin-V positive MCF-7 cells. These results were confirmed by data of DNA laddering assay showing a clear evidence of nucleosomal DNA fragmentation in VA-treated cells. No statistically significant was recorded with regard to caspase 3 activity in MCF-7 cells, probably due the fact MCF-7 does not express caspase-3 protein. Taken together, our research demonstrated that VA represents an apoptosis-inducing agent and its apoptotic mechanisms involve phosphatidylserine externalization, and nucleosomal DNA fragmentation. However, a large proportion of cell death was by necrosis as revealed by the propidium iodine assay.

**Keywords**: *Vernonia amygdalina*, MCF-7 cells, breast cancer, cellometer vision, flow cytometry

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