Vernonia amygdalina - Medicinal Uses and Anti-cancer Effects of Nigerian Bitter Leaf

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Vernonia Amygdalina, also known as bitter leaf or VA, is a shrub that grows 2-5 meters high and is native to Nigeria. The plant has been highly researched for its medicinal properties and apparent anticancer biological effects. It was discovered by researchers interested in the lives of chimpanzees in Nigeria, who were observed chewing on bitter leaf plants when suffering from parasitic infestations. (7) Research has been ongoing since the mid 1960's about the health benefits of VA and the mechanisms by which it works. (11) It plays an important role in African ethnomedicine, essentially being used as a panacea to treat a variety of illnesses including gastrointestinal disorders, diabetes, malaria, and most notably cancer. The mechanisms behind each of these biological activities can likely attributed to a variety of different pathways. Our paper will focus on the anti-cancer properties of bitter leaf by investigating the mechanisms which make this plant appear to be so effective at combating this disease. We will also seek to unearth any potentially detrimental effects of the plant and the various phytochemicals which are its constituents. Finally, we will explore the potential impacts of the use of VA as an anti-cancer treatment, as well as beneficial effects it may have when used in the treatments of other maladies.

Several key questions we address in the course of our research include: What is the mechanism/pathway used to makes this plant effective as an anti-cancer agent in the human body? Does VA a similar pathway or mechanism in its function as an effective treatment for malaria and worm-type pathogens? If not, what other mechanisms does it use? Finally, are there potentially detrimental effects of ingesting VA or using a VA derivative as a medical treatment for cancer? We use these questions to

outline the scope of our paper and to guide our research as we compile data on VA and synthesize many semi-disjointed studies into a more basic understanding of the plant and its possible applications as an anti-cancer drug.

Although Vernonia Amygdalina has shown promising potential as an anti-cancer agent, further research needs to be done to determine specifically which mechanisms VA uses to produce these effects. Phytochemicals found in VA are known to have numerous biological effects, which combine in such a way that the biological effect is cancer chemoprevention. A number of studies have shown VA to effectively slow the proliferation of breast cancer cells and also inhibit growth promoting pathways between cells. These are common characteristics of the mechanisms used by many anti-cancer agents. Much of the research done on VA up to this point has been aimed at comparing VA to current anti-cancer agents, and observing parallels between them. Drug pathways are not always entirely known or understood, so it is not certain whether the observed similarities are significant to the drug's effect on cancer treatment but certainly suggest promising potential for the use of VA as a chemotherapy drug.

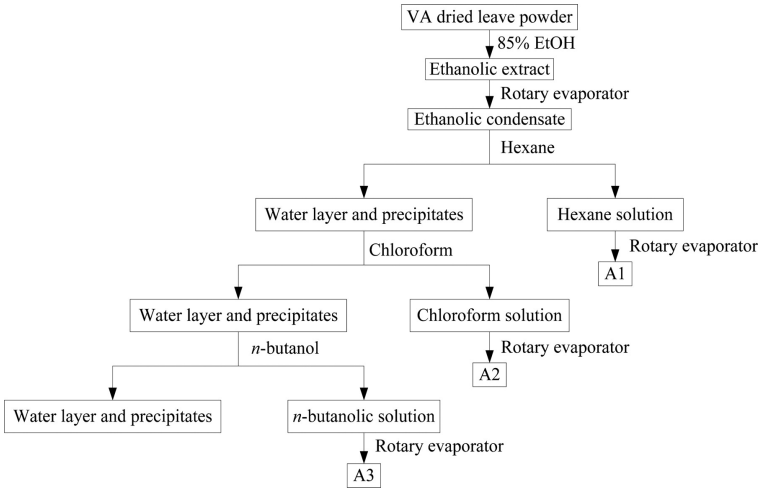


Figure 1. Denotes the variety of solutions that can be made from VA leaf powder, each with different effects on the proliferation of cancer cells. The fact that so many solutions can be made and tested

shows that synthesis of an anti-cancer drug from VA is a complex process which has only recently entered testing phases.

ERK Inhibition (DNA synthesis inhibitor):

One of the shared characteristics of the mechanism of many anti-cancer drugs is activity inhibition of extracellular signal-regulated kinases (ERKs). ERKs serve to convert stimuli outside of cells to regulatory signals within cells, specifically signals regulating the expression of genes that are critical to cell growth and differentiation. It comes as no surprise that raised levels of ERK expression is associated with breast cancer. Due to this observation, a team of researchers decided to look into the interaction between VA and ERKs, to determine whether VA operates in a similar way to these ERK inhibiting anti-cancer agents. In this study, researchers tested the thymidine kinase incorporation in cells treated with aqueous extracts of VA, and found a dramatic concentration-dependent decrease in the viable cell number in the VA-treated cells. Thymidine kinase is an enzyme that is only present in anticipation of cell division. It used in clinical chemistry as a proliferation marker, specifically as a marker of malignant cell growth (11). Increased dosage of water soluble extracts of VA led to decrease in uptake of thymidine kinase, meaning an inhibition of mitosis in VA treated cells. This study essentially showed that VA functions as a DNA synthesis inhibitor, which has significant implications for the use of VA as a potential cancer treatment (6).

Another characteristic of several known anti-cancer agents is the induction of increased expression of phase 2 metabolic enzymes, without effecting phase 1 enzymes. Phase 1 metabolic cytochrome enzymes (CYP) are involved in oxidative metabolism, and it has been suggested that impaired oxidative metabolism may cause malignant growth (10). A study was done to investigate

whether VA behaved in a similar way to the anti-cancer agents, regarding interaction with metabolic enzymes. Exposure of cells to low doses of VA did not affect expression levels of phase 1 CYP enzymes, but lead to induction of phase 2 microsomal epoxide hydrolase (mEH), thus supporting the chemotherapeutic potential of VA. (4).

Along with cellular viability, VA has shown to cause minimal genotoxic damage in MCF-7 tumor cells. There was an observed increase in comet tail-length, tail arm and tail moment, as well as in percentages of DNA cleavage in VA-treated MCF-7 cells. Although agents which damage cells but do not lead to cell death can be possible mutagens and/or carcinogens, it has been reported that agents that cause minimal DNA damage are typically effective in cancer treatment. VA is similar to known anti-cancer agents in its ability to both induce phase 2 metabolic enzymes, and cause minimal DNA damage to tumor cells, (12).

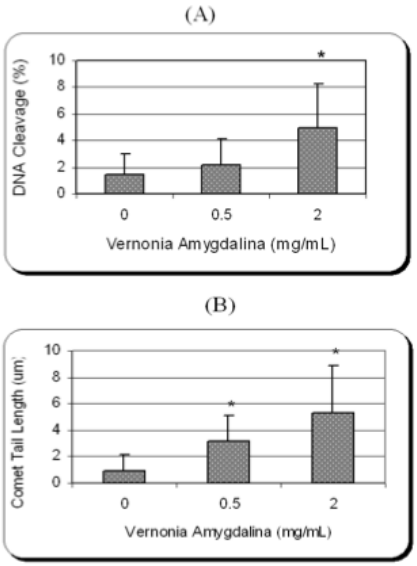


Figure 3: Comet assay of MCF-7 cells showing the percentage of DNA cleavage (Left) and tail length (Right), as a function of VA leaf extracts doses. Each point represents mean ± SD of 3 independent experiments. *Significantly different ($p < 0.05$) from the control, according to the Dunnett's test.

The figure above shows the correlation between increased concentration of VA extract used to treat

MCF-7 cells, and a subsequent increase in both comet-tail length and percentage of DNA cleavage as a result.

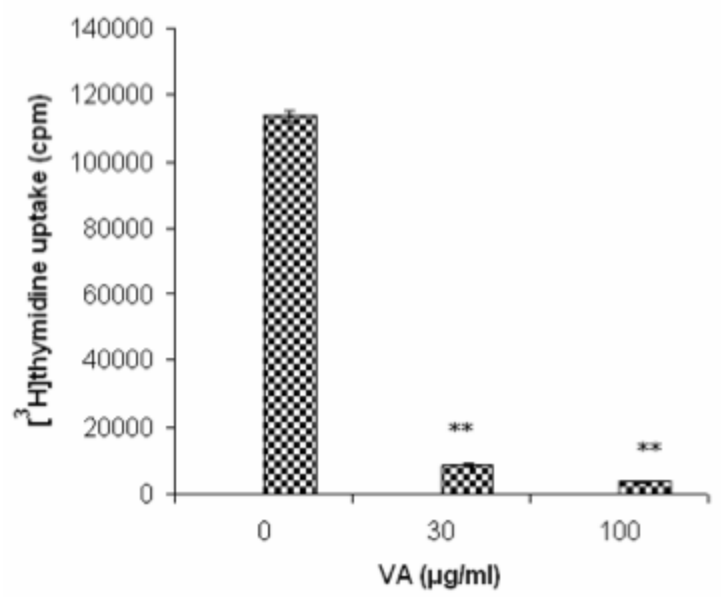
Blocking Estrogen Receptors

Some breast cancer tumors require estrogen for continued proliferation and can be identified by the presence of estrogen receptor on their surface. ER+ cancers are treated with drugs that either block the receptors or block the production of estrogen. Previous reports show that low concentrations (microgram/ml) of water-soluble leaf extracts of VA potently retards the proliferative activities of ER+ human breast cancerous cells (MCF-7) in vitro in a concentration-dependent fashion through the suppression of Aromatase activity (1). Aromatase is an enzyme that catalyzes the reaction in which testosterone is converted to estrogen. This is the same mechanism utilized by many of the drugs used to treat breast cancer. Although these tests were all done *in vitro,* they show promising potential for the use of VA *in vivo* for tumor stabilization or preventative treatment (1) .

There is hope for VA as an effective treatment for ER- breast tumors as well. Evidence suggests that most chemotherapeutic agents are less effective as treatment in patients with estrogen receptor-negative (ER-) breast carcinomas compared to those with estrogen receptor-positive (ER+) breast carcinomas. Novel therapies effective against ER- breast carcinomas are urgently needed to ameliorate the health disparity between ER+ and ER- breast cancer patients. The anti-proliferative activities of VA in either ductal or ER- carcinoma cells have not yet been characterized, however, the exposure of the ER- breast cancer cell line BT-549 to increasing concentrations of VA (10, 100, and 1000 μg/mL) inhibited cell growth by approximately 14% ($P<0.05$), 22% ($p<0.05$), and 50% ($p<0.005$) respectively. (2).

Thionins

Aqueous VA alters cell permeability in two ways. It limits the cell's ability to take in nutrients as well as its ability to retain those nutrients. Cell culture and animal model studies have revealed that the a specific component of the plant extract called thionins are partially responsible for the plant's anticancer properties (9). Thionins are a cytotoxic molecule present in a number of species of plants as means defense against consumption by animals, bacteria, and fungi. Thionins are believed to interact with the cell's membrane in a way that causes it to become permeable, inhibiting nutrient uptake and allowing essential nutrients and ions to leave the cell which leads to apoptosis. The mechanism for how VA induces apoptosis in cancerous cells is a topic of ongoing research, yet it is known that this affect is concentration-dependent. In an experiment done by MM Opata, it was shown that exposure of cells to VA decreased [3H]thymidine uptake to a greater extent as the concentration of VA increased (0, 30, and 100 mug/mL, $p< 0.05$) (9). Thymidine is one of the components of DNA and therefore is crucial to cell replication. Concentration also affected [3H]thymidine release in a positive correlation. The amount of [3H]thymidine released into the medium was 1.7, 7.4, and 11.0% for 0, 30, and 100 mugl/mL respectively ($p<0.05$) (9). Further thionin research is needed to determine all of the applications of thionins from VA and other thionin producing species.



(9)

Detrimental Effects

Initially researchers were concerned about potential harmful effects of VA on the body, considering its widespread effects when ingested or synthesized into traditional ethnomedical elixirs. Specifically, research had to be done about the potential toxicity of VA due to the fact that long-term effects of the chemicals found in the plant were largely unknown. The effects of various concentrations of aqueous extract of Vernonia amygdalina leaves on some biochemical indices of liver function were investigated in albino Wistar rats. The results of the study strongly suggest that V. amygdalina leaf extract is not hepatotoxic in rats, meaning it essentially has no harmful effects on the liver and therefore is not remarkably toxic. These findings "are of nutritional, clinical and veterinary relevance considering the diverse applications of the plant in almost all African populations." (8)

Though the potential detrimental health effects of VA are not widely known, the implications of its widespread use in traditional medicines suggests that it has few negative effects which have been discovered. The fact that it is commonly ingested by chimpanzees and humans suggests that it is fairly

safe to eat, or at least that its medicinal benefits outweigh the as-yet undocumented costs it may involve. Herbal remedies are believed by the general public to be substantially safe anti-cancer agents, as they typically cause less side-effects and are less likely to cause dependency. However, this also inherently bears a link to them being less effective as medical treatments as well. Therefore, it is interesting to note that VA seems to be extremely valuable as an effective anti-cancer agent with few harmful side effects, even though years of further research on these two topics still must be done before this plant and it's potential applications are understood fully. The general consensus on VA given the substantial amount of research that has been performed seems to be that water-soluble Vernonia amygdalina extract treatment inhibits cancerous cell growth without harmful side effects to normal cells (3).

Discussion and Concluding Remarks

Vernonia Amygdalina is a promising new option for chemotherapy, as well as a variety of other maladies. VA has shown to be extremely versatile and complex in both mechanism and function. Hopefully the curiosity surrounding the potential of VA will fuel further research so that it can begin to be utilized by health care providers. The demand is high for alternatives to current chemotherapy techniques, so it is imperative that options like VA are thoroughly investigated in order to move towards viable solutions. The question of whether VA is effective against ER- breast carcinomas is of particular interest, as there are few current treatments available. Research on how the presence of VA interacts with ER- tumors would prove extremely valuable if the addition of VA to a chemotherapy regimen would help combat this therapy-resistant type of carcinoma. Experiments to determine how VA increases sensitivity to chemotherapy would also help to answer questions such as: would the addition of VA into mainstream diets have a positive impact on the general populous or should it be specifically

given to cancer patients? Could VA add to the effects of modern drug-based therapies? Or could VA be combined with another plant-based medicine to increase its effects?

Investigating whether VA could operate as a chemotherapy enhancement drug is another question that researchers have started to look into, but certainly needs to be explored more. VA is an encouraging possibility for a potent drug with, in most cases, seemingly negligible detrimental side-effects. As stated by Ijeh et. al, "Future research must aim at characterizing the active principle(s) responsible for each effect, and determining if they act singly or synergistically with other principles present in the plant. Only such research would place V. amygdalina Del. in its proper place in nutritional and medical sciences". (5)

Application for humans also include the addition of VA to mainstream diets, if the benefits of VA could be incorporated into human ingestion on a regular basis. It would be interesting to find out if it can be eaten in raw form, or if synthesis as a pill or liquid extract would be easier to eat. It is possible to suggest that VA and chemotherapy combined may provide a cost-effective way to treat cancer, though it must be remembered that alternative cancer treatments like VA are not a substitute for modern medicine. However, it is safe to say that the potential impacts on anti-cancer and anti-malarial drug treatments that VA contains are uplifting and deserve to be studied further. Perhaps a cure for cancer could one day be synthesized from plants like VA.

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