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Mechanisms of Anti-Cancer Effects of *Vernonia amygdalina* Leaf Extract

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Abstract: Problem statement: *Vernonia amygdalina* (*V. amygdalina*) has been shown to have cancer cell inhibition and cytotoxic effects. **Approach:** This study discusses the multi-faceted and multi-linked mechanisms by which cancer tissue inhibition and destruction is achieved by *V. amygdalina* leaf extract and powder (subsequently called *V. amygdalina* Extract). Cancer cell inhibition by *V. amygdalina* is suggested to occur through inhibition of sterol 14-α-demethylase, a microsomal P450-dependent enzyme system) of the membrane of the cancer cell. Inhibition of this enzyme impairs the biosynthesis of ergosterol for the cytoplasmic membrane. **Results:** This impairment of the synthesis of ergosterol disrupts the close packing of acyl chains of phospholipids and impairs the function of some membrane-bound enzyme systems like ATPase and enzymes of electron-transport system of the cancer cells. *V. amygdalina* extract inhibits sterol 14-α-demethylase by acting on β_3 adrenergic receptors of the cancer cell membranes as a primary ligand in Gi (inhibitory) receptors on cancer β_3 lipid cell membrane receptors and mitochondrial ATP energy generation system receptors. **Conclusion:** By effecting β-oxidation of fatty acids and lipids in cell membranes and cell mitochondrial energy (ATP) generation systems, the *V. amygdalina* extract uncouples the mitochondrial energy (ATP generation) systems of the cancer cells and cell membranes resulting in the cancer cells being burnt out (consumed) by the extract (when *V. amygdalina* extract is cytotoxic to the cancer cells) or its functioning being inhibited (when *V. amygdalina* extract is only inhibitory to the cancer cells.

Key words: ATP generation, cytoplasmic membrane, anti-carcinoma, *Vernonia amygdalina*, leaf extract, cardiac glycosides, sesquiterpene lactones, immune defence

INTRODUCTION

V. amygdalina contains saponins, cardiac glycosides, flavonoids and sesquiterpene lactones.

Its major constituents are the saponin Vernonin, the sesquiterpenes Vernolepin and Vernodalin and the ubiquitous flavonoid Kaempferol.

Its sesquiterpene lactones have in-vitro cytotoxic activity against KB tumor cells and Wilme's myeloma and its sesquiterpene lactone prevents arterosclerosis.

Its leaves are reputed to be effective remedy as a general tonic, for gastrointestinal disorders and for fevers and its leaf extract is taken for diabetes reduction.

Its bark is administered for the treatment of venereal diseases and its leaves are ingredients in purgative enemas, diuretic mixtures, anthelmintic preparations and topical lotions for parasitic diseases. The organ and medicinal effects of *V. amygdalina* have been demonstrated in various studies (Oyugi *et al*., 2009; Lam *et al*., 2011).

Anti-cancer activities of *V. amygdalina* have been reported in many studies (Oyugi *et al*., 2009).

Anticancer effect of *V. amygdalina* leaf extract is an immune defence action: The position of the author is that the carcinoma inhibition and destruction actions of *V. amygdalina* extract stem from a combination of its cellular energy generation antagonism and its immune defence actions. The Immune Defence actions of *V. amygdalina* include all those actions it carries out in defence and protection of the various organs of an organism. The action of identifying cancerous tissues of an organism as a "non-self" and as detrimental to the life of the individual is in itself a non-specific immune response (Hyde and Benbrook, 2006; Suzuki *et al*. 2005). The inhibition and destruction of carcinoma by *V. amygdalina* extract are thus clearly immune defence actions as they rid the body of unwanted malignant cells. *V. amygdalina* leaf extract has been shown to have immunomodulatory properties (Barret and Blanc, 2009) Antimicrobial (Alabi *et al*., 2005), antiparasitic,

antioxidant, anti-inflammatory, free scavenging, peroxidation and wound healing actions of *V. amygdalina* leaf extract (Lu *et al*., 2010; Arhoghro *et al*., 2009; Esimone *et al*., 2005) are all extensions of the immune defence actions of the extract.

Lipid-lowering, anti-oxidant and anti-diabetic effects of *V. amygdalina* extract as partners in the anti-carcinomer effects of the extract: The lipid-lowering or lipolytic effects of *V. amygdalina* created holes in the cell membranes of the malignant cancer cells causing them to burst or lyse. The presence of such holes also makes it easier for phagocytes and nonspecific immune defence scavenging leucocytes of the patient to destroy such cells. Antioxdant and peroxidation effects of *V. amygdalina* leaf extract which have been demonstrated in many studies essentially works simultaneously with its lipid lowering actions and its anti-hyperglycaemia effects to inhibit and eliminate cancer cells.

Cancer Inhibitory actions of *V. amygdalina* extract: In a study, extraction of *V. amygdalina* leaf with multiple solvents of various polarity indexes yielded three fractions that significantly ($P < 0.05$) inhibited breast carcinoma cell growth at 0.1 mg mL^{-1} concentration; and at a higher concentration of 1 mg mL^{-1} yielded 6 fractions of hexane, chloroform, butanol and ethyl acetate which inhibited DNA synthesis by 76, 94, 98, 98 and 96% respectively (Oyugi *et al*., 2009). The 94-98% inhibition of DNA synthesis demonstrated in this study by 5 out of 6 solvent fractions of *V. amygdalina* suggests that inhibition of DNA synthesis is the major mechanism of cytotoxic action of *V. amygdalina* extract. In the bioactivity assays of breast carcinoma inhibition by *V. amygdalina* in the same study, HPLC retention time of approximately 2 min were found to be required for cell growth inhibitory activity of *V. amygdalina* fraction (Oyugi *et al*., 2009).

The mechanism of cancer tissue-inhibition and cancer tissue-cytotoxic effects of *V. amygdalina* leaf extract: The studies on anti-breast carcinoma cell growth fractions of *V. amygdalina* has shown that 3 fractions of *V. amygdalina* of multiple solvents each significantly ($p < 0.05$) inhibited cell growth at a concentration of 0.1 mg mL^{-1} (Oyugi *et al*., 2009). Six other fractions of different solvents inhibited DNA synthesis at a concentration of 1mg mL^{-1}, in the same study. The percentage of inhibition of DNA synthesis by these 6 fractions was 76, 94, 98, 98, 96 and 98%. The 94-98% inhibition of DNA synthesis was obtained with *V. amygdalina* extract fractions of five different solvents showed that the 76% inhibition obtained with the sixth solvent must have been due to experimental error. A 94-98% inhibition of DNA synthesis in any living cell will definitely grind the activities of that cell to a halt. Such paralysis of a cell will definitely destroy the cell.

The difference between the DNA-inhibiting fractions and the cell-inhibiting fractions of *V. amygdalina* leaf extract in the breast cancer study was the strength or concentration of the extract. The strength of the DNA-inhibiting fraction was ten times that of the cell-inhibiting fraction (1 mg mL^{-1} as opposed to 0.1mg mL^{1}). The nature of the solvent was not an important factor in the manifestation of the cell or DNA-inhibiting effects of *V. amygdalina* leaf extract in the study.

The results of this breast cancer study (Oyugi *et al*., 2009) suggests that DNA synthesis inhibition and cell membrane disruption effects of high concentrations of the *V. amygdalina* extract are necessary for cancer cell cytotoxicity by the extract.

V. amygdalina produced significant inhibition of acetic acid induced writhing and the formalin test in mice. An infusion of *V. amygdalina* leaves reduced the haemolysis of human erythrocytes *in vitro* in a study in which human genotype-SS was highly susceptible to haemolysis (1024), human genotype-AS, moderately susceptible (512)and human genotype-AA highly resistant (256) *V. amygdalina* protected alluminum against acidic corrosion and had inhibition efficiency of 49.55 for 0.1M Hcl and 72.5% for 0.1M HNo_3 respectively against acidic corrosion of 2S and 3RS alluminium alloys. *V. amygdalina* extract also produced significant 73% inhibition of plasmodium berghei parasitaemia in mice in the group that received a dose of 200 mg kg^{-1} I.P (intra peritoneal) for 4 days value of *V. amygdalina* extract = 112 2 mg kg^{-1}. These cited examples illustrate that *V. amygdalina* extract uses its inhibitory effects to protect the integrity or wellbeing of a host organism or even a non-living organism like alluminium. This explains why *V. amygdalina* leaf extract employs its inhibitory and ati-oxidant effects to protect an individual whose tissues have become cancerous.

The energy metabolism inhibition effects of *V. Amygdalina* extract: The energy metabolism inhibition effects of *V. amygdalina* utilized in its anticancer actions are exerted on the cell membrane, on DNA and on the cytoplasm of the cancer cells. These energy metabolism actions start with lipolytic effects on cell membranes and lipid depots of carcinoma cells and

extend to the excess body fat in the adipose tissues of a cancer patient.

If hyperglycaemia is present in the cancer patient, the *V. amygdalina* extract will also exert a hypoglycaemic action. The high efficacy of *V. amygdalina* leaf extract in blood glucose lowering is reported by many studies. Blood glucose lowering is the first step in the inhibition of the energy metabolism of the cancer cell.

The high efficacy of the lipid lowering actions of *V. amygdalina* has also been demonstrated in some studies

The uncoupling of ATP and metabolism by *V. amygdalina* leaf extract: *V. amygdalina* extract inhibits cancer cells by inhibiting their cellular energy generation sources. This action is started in the mitochondria of fat cells (especially brown fat adipose tissues) where there is the usual inward proton conductance that generates ATP (oxidative phosphorylation) but there is also a second short circuit proton conductance that does not generate ATP

This second short circuit proton conductance which does not generate ATP is associated with a polypeptide of molecular weight 32,000 in the membrane, which causes uncoupling of metabolism and generation of AT P so that more heat is produced .When the ATP and energy metabolism- uncoupling actions of high concentrations of *V. amygdalina* extract are exerted on the double lipid membrane of cancer cells and on their mitochondrial oxidative phosphorylation, these high concentrations of the extract inhibit the DNA of the cancer cells, burn out their cell membranes and kill the cancer cells. This uncoupling of the energy source of cancer cells was observed as 98% inhibition of DNA synthesis by *V. amygdalina* leaf extract in the breast cancer cells inhibition study (Oyugi *et al*., 2009).

Stimulation of sympathetic innervations to brown fat releases noradrenaline which acts on B_1 adrenergic receptors to increase lipolysis; increase fatty acid oxidation in the mitochondria and to increase heat production. Transgenic rats in which brown fat is ablated become obese Nerve discharge to brown fat is increased after eating so that heat production is increased.

The cancer cell destruction effects of high efficacious concentrations of *V. amygdalina* Leaf extract are exerted by the extract acting as a primary ligand in Gi receptors on cancer β_3 lipid cell membrane and cell mitochondrial ATP (energy) generation system receptors. By inhibiting and thus uncoupling the mitochondrial energy (ATP generation) systems of the cancer cell membrane and cytoplasm, the cancer cell is denied the energy to power DNA synthesis and subsequent cell survival/growth and its cell membrane/ytoplasm become consumed by the *V. amygdalina* extract.

Secondly, low efficacious concentrations of *V. amygdalina* inhibit certain membrane-bound enzyme systems like adenosine triphosphatase and electron transport systems by inhibiting sterol 14-α-demethylase that catalyses the biosynthesis of ergosterol for the cytoplasmic membrane. This inhibition of the membrane enzyme systems of the cancer cell inhibits the growth of the cancer cell and can cause the perforation and lyses of some of the cancer cells.

A study found that the wild type bacteriophage T_4 (wtTw) and its substrain HAP 1 had enhanced affinity for melanoma cells by 47 and 80%, respectively and suggested interaction between the Lys-Gly-Asp motif of the phage protein 24 and β_{3-} intergrin receptors on target cells (Galustian *et al*., 2008, Suzuki *et al*., 2005). This study also showed that anti- β_3 antibodies and synthetic peptides mimicking natural β_3 ligands inhibit the phage binding to cancer cells (Galustian *et al*., 2008, Suzuki *et al*., 2005)

The findings of this study support the position of this author that β_3 adrenoceptors of carcinoma cell membranes and cytoplasmic/mitochondrial energy systems are the receptors on which *V. amygdalina* leaf extract acts to produce its carcinoma inhibition and destruction (cytotoxicity) effects.

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