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editor@iajavs.com
iajavs.editor@gmail.com

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The Effects of Vernonia amygdalina on Vascular Smooth Muscle Cell Proliferation and Cytotoxicity in HT 29 Cell Lines

Miss. V. Prasanna Vaishnavi[1], Mrs. A. Deepthi[2], Mrs. S. Shaheentaj[3], Miss. P.S. Chandana[4], Mr. A. Sreedhar[5], Mr. N.V. Murali Krishna[6]

ABSTRACT

Using the Cell Titer 96 MTT Proliferation Assay, the antiproliferative effects of methanol leaf extracts of Vernonia amygdalina (V. amygdalina) Del (Asteraceae) on Vascular Smooth Muscle Cells (VSMC) and Human Colorectal Adenocarcinoma Cell line (HT 29) were examined. The extract was administered to VSMC at log doses of 200 and 800 g/mL and incubated for 24 and 48 hours. Extracts were also incubated for varying amounts of time with VEGF and ET-1 (the mitogens). The HT 29 cell line was treated with varying concentrations of extract (200, 400, and 700 g/mL) for 24, 48, and 72 hours. After 24 hours, both the plant extract and the mitogens had a greater impact on VSMC proliferation than the plant extract alone or in the presence of mitogens (except at 200 g/mL, when there was no proliferation in the presence of ET-1). The increase in growth was much more noticeable after 48 hours. Increase of 153.3% was seen, for instance, when 800 g/mL of V. amygdalina was combined with VEGF. In the cytotoxic trial with HT 29, the extract was cytotoxic throughout the whole dosage range. At 72 hours, 69.2% of cells were inhibited by a V. amygdalina dose of 200 g/mL. However, it should be pointed out that the extract's impact was most evident and persistent at the 24-hour time point. From these results, we may infer that although the extract stimulates VSMC proliferation, it has the opposite impact on the HT 29 cell line. Since the extract was shown to have inhibitory effects on the HT 29 cell line, this suggests that it displays a cytotoxic impact and might potentially serve as lead agents in the quest for an anticancer medication derived from natural sources.

Keywords*: V. amygdalina; HT 29; VSMC; anticancer; VEGF; ET-1.*

1. INTRODUCTION

The identification of substances that may either slow or speed up cell division is crucial in the fields of cell biology and drug development. More than 25% of medications during the last few of decades have come from plant species, and another 25% have been chemically modified from their original state. About 5-15% of the 250,000 or more higher plants have had their bioactive chemicals researched [1,2].

New and unique products from possible bioactive plants or their extracts for disease treatment and prevention are still enormous [3], despite the fact that synthetic chemistry has developed as a technique of drug discoveries and drug manufactures in recent years. The virtually infinite potential of plants to produce chemicals that interest scientists in their hunt for novel chemotherapeutics [4].

Cancer is one of the worst illnesses people may get, but scientists have recently made important discoveries in the development of novel anticancer drugs derived from natural chemicals [5]. Surgery, radiation therapy, immunotherapy, and chemotherapy are all alternatives for treatment, but they are either too hazardous, too costly, or both. This prompted the research of potential herbal treatments with anti- Cancerous behavior. Natural compounds from plants have demonstrated great promise in reducing cancer while presenting few or no side effects, and medicinal plants have long been utilized to treat a wide range of chronic conditions. This is why plant extracts are becoming more popular as an alternative to conventional medication. In reality, because to a lack of access to allopathic medication [6-9], as much as 80% of the population in poor nations relies on herbal therapy to cure various ailments.

Department of Pharmaceutics[1,2,3,4,5], Pharmaceutical Analysis[6]
Approved by AICTE& Pharmacy Council of India, New Delhi.(Affiliated to jawahalal Nehru Technological University. Anantapur&S.B.T.E.T.A.P)
Chennai-Hyderabad By Pass Road, Ukkayapalli, Kadapa-516002

While chemotherapy may prolong a cancer patient's life by a few years, it comes with the danger of fatal host toxicity and other major side effects [10]. There is a growing interest in and understanding of the fact that chemotherapeutic medicines work largely by causing cancer cell death through the process of apoptosis, and there is a corresponding desire for the employment of alternative ideas or tactics for cancer prevention. Many tumors, however, are genetically programmed to resist apoptosis, making it imperative to create new medications for combination chemotherapy [11,12]. Natural extracts' chemo-protective potential may be better understood by measuring their anti-proliferative effects on cancer cells using the MTT test.

Chemoprevention relies heavily on early-stage cancer eradication [13].

The Asteraceae family member Vernonia amygdalina Del is used for its therapeutic properties, and it is found over most of East and West Africa [14,15]. Bitter leaf, also known as berberine, has potential as a cancer-fighting agent [16], as well as against germs, malaria, and parasites [17]. The plant's complex active components have therapeutic use in pharmacology. Both the roots and the leaves are used in traditional medicine [18,19] to alleviate symptoms such as fever, hiccups, renal issues, and abdominal pain.

Vernonia amygdalina chloroform extract was originally found to have an anti-cancer effect in human carcinoma of the nasopharynx [20,21]. This effect was subsequently confirmed in leukemia cells P-388 and L-1210. Water-soluble leaf extracts at low concentrations (g/mL) significantly inhibited the proliferation of estrogen receptor positive (ER+) human breast cancer (MCF-7) cells in vitro, as shown by research by Izevbigie [16]. Treatment with Vernonia amygdalina- has been demonstrated in other research [22] to affect phase 1 and phase 2 gene expressions in MCF-7 cells in a time- and dose-dependent manner. The purpose of this investigation was to assess Vernonia amygdalina's (VA) methanol leaf extract's (VE) effects on vascular smooth muscle cells (VSMC) and the HT29 cell line.

2. MATERIALS AND METHODS

2.1 Collections and Identification of Plant Materials

Fresh leaves of *Vernonia amygdalina* were collected from the community around the University of Ibadan campus and then subsequently authenticated at the Department of Botany University of Ibadan where specimen voucher was also kept. The herbarium number is UIH-22640.

2.2 Preparation of Plant Extracts

2.2.1 Methanol leaf extraction of *Vernoniaamygdalina*

The fresh plant leaves were washed with waterto remove dirt and then dried under shade. The leaves were pulverized into powder form and 200g of the powdered materials were separately dissolved in 2 L of methanol under cold maceration. After 72 hrs, the solution was filtered using Whatman filter paper. The black material in a slurry form was then placed in porcelain plate and put on hot water at about 40ºC to removethe remaining methanol in the extract.

2.2.2 MTT assay

Cells were cultured to confluence, trypsinized and plated in 96-well plates for cell proliferation assay. Twenty four hours after plating, cells were treated with various concentrations (25-100 µg/mL) of the extract along with the control in thepresence or absence of mitogens and cultured for 24-48 hours to determine effects of treatment on cell growth.

MTT assay is based on the ability of cell to reduce MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide to purple formazan in the mitochondria of living cells. The viable cells (VSMC and HT 29) were seeded at a density of 5×10^4 (100 μL/well) in 96-well plates and incubated in a humidified atmosphere of 5% $CO_2$and 95% air at 37ºC for 24 h to form a cell monolayer. MTT assay was performed over three days. On day one, the cells were trypsinized. After these have confluenced and on the second day, the cells were treated with *Vernonia amygdalina*, mitogens and *Vernonia amygdalina*
+ mitogens and the final volume of the media was adjusted to 100 µL and the incubation continued. On day three, 20 µL of 5 mg/mL of MTT was added to each of the 96 wells but the well used, as controls have no cell. This wasthen

incubated for three and half hours at 37ºC in culture hood. After this, the media was carefully removed and 150 µL of MTT solvent was added and covered with tin foil and cells agitated on orbital shakers for 15 minutes. Thereafter, the absorbance was read at 590 nm.

2.3 Statistics

Results are expressed as mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA), using Graph Pad Prism version 6. The level of statistical significance wasconsidered as $\alpha<0.05$.

3. RESULTS AND DISCUSSION

3.1 Effects of the Extract of *V. amygdalina* on VSMC

The result of this study showed that after 24 hours, the effect of the extract on the VSMC alone and in the presence of the mitogens was more of proliferation. For instance, the 200
μg/mL of the extract alone and in the presence of VEGF caused respectively 25 and 108.3% increase in cell proliferation but in the case of the 800 μg/mL alone and in the presence of VEGF the result was 83.3 and 41.7% increase in cell proliferation showing that in a way there is reduction in cell proliferation at this dose in the presence of VEGF. The result also showed that the extract at 200 μg/mL in the presence of ET-1 caused no cell proliferation whereas the 800
μg/mL in the presence of ET-1 caused 33.3% cell proliferation indicating that this mitogen also interfere with the extract's cell proliferation activity. At 48 hrs, the 200 μg/mL alone caused 73.3% increase in cell proliferation but in the presence of VEGF and ET-1 it caused 86.7 and 80% increase in cell proliferation. With respect to the 800 μg/mL alone and in the presence of VEGF and ET-1, there was increase in the proliferation of the cells at 126.7, 153.3 and 100%, respectively (Figs 1 and 2).

Studies with natural products involve biological activity and pharmacological assays for the screening of plant extracts, with subsequent evaluation of the fractions and isolated compounds were responsible for the activity observed [23].

In this present study *Vernonia amygdalina* alone and in the presence of VEGF resulted in a dose dependent and time dependent increase in cell proliferation which is similar to what Parenti et al. [24] demonstrated that VSMC proliferation induced by monocyte chemoattractant protein-1 was mediated by endogenous production of VEGF-A.

Vasoactive agents such as norepinephrine (NE), angiotensin II (ANG II), and endothelin-1 can induce hypertrophy and proliferation of vascular smooth muscle cell and thus are implicated in pathogenesis of hypertension [25-28]. Endothelins (ETs) are a family of three peptides (ET-1, ET-2, ET-3) that are implicated in the physiological control of vascular smooth muscle cell (VSMC) and myocardial contractility and growth. ET-1 is vasoactive peptide that acts via ET-A receptors coupling inducing vascular smooth muscle cell contraction [29-31].

In this study at 24 hours, the 200 μg/mL dose of *Vernonia amygdalina* resulted in an inhibition of the proliferation of VSMC in the presence of ET-1, the higher dose (800 μg/mL) however showed a slight increase in cell proliferation. At 48 hours, however, there was an increase in theproliferation of the VSMCs in the presence of

both *Vernonia amygdalina* and *Vernonia amygdalina*+ET-1. It can be deduced from this observation that the proliferative effect of the plant on VSMC is dependent on time and dose. VSMCs have been viewed as directly responsible for generating the atherosclerotic plaque, via proliferation, migration from the media and synthesis of matrix proteins [32]. Consequently huge efforts have been made to inhibit the accumulation of these cells, which in the case of stent stenosis have largely been successful. However, reviews of atherosclerosis have emphasized the advantageous protective function of VSMCs in atherosclerosis [33]. This is based on findings that plaques in humans that have undergone plaque rupture, and directly led to heart attacks, show a paucity of VSMCs compared with stable lesions [34]. Indeed VSMCs are the only cells capable of synthesizing components of the fibrous cap in plaques (the structure that separates the blood from the thrombogenic plaque interior), and whose rupture or erosion may trigger myocardial infarction.

Apoptosis of VSMCs has been recognised in atherosclerosis. In early lesions, apoptotic frequencies are minimal but peak in advanced plaques with both VSMCs and macrophages showing features of apoptosis [35]. This observation raised the possibility that VSMC apoptosis could promote plaque rupture by thinning the fibrous cap. Indeed, plaques from patients with unstable symptoms show higher levels of apoptosis than those with stable lesions [36]. VSMC apoptosis has also been associated with numerous other features within plaques including inflammation, calcification, thrombosis
[37] and both negative remodeling (vessel shrinkage) [38,39] and aneurysm formation [40]. VSMC apoptosis causes release of IL-1 and up-regulation of monocyte-chemoattractant protein 1 (MCP-1) [41] and interleukin (IL) 8, causing infiltration of macrophages in vivo [42]. *In vitro*, VSMC apoptosis can promote both thrombin generation [43] and vascular calcification [44], and apoptotic vascular cells are thrombogenic both locally [45] and systemically [46]. Therefore the proliferation of VSMC by *Vernonia amygdalina* at higher dose in this study might be a useful therapeutic target to prevent VSMC apoptosis in stable and unstable atherosclerosis.

3.2 Effects of the Extract of *V. amygdalina* on HT 29 Cell Line

In the case of HT 29 cytotoxic study, at 24 hrs, the 700 μg/mL concentration caused the greatest cytotoxicity at 61.8% cell inhibition and this was followed by the 400 μg/mL concentration at 60.5% decrease in proliferation. The 200 μg/mL concentration of the extract only caused 52.6% inhibition of the HT 29 cell line. At 48 hrs time point, the 200 μg/mL concentration caused 50% inhibition followed by that of 400 μg/mL causing inhibition at 37.5% while the 700 μg/mL caused 12.5% cell inhibition. At 72 hrs, the 200 μg/mL concentration caused the greatest cytotoxic effect at 56.1% cell inhibition and this is followed by 400 and 700 μg/mL at 51.2 and 31.7%, respectively. It is to be noted that the antiproliferative effect of the extract increases with time with the 200 μg/mL concentration showing some form of consistency with time (Figs. 3 and 5).

It has been known that plants have a long history of use in the treatment of cancer [47] and herbal medicines have a vital role in the prevention and treatment of cancer [48]. The use of plant derived natural compounds as part of herbal preparations and alternative sources of drugs continues to play major roles in the general wellness of people all over the world [47,49]. Agents capable

4. CONCLUSION

The present data demonstrate that *Vernonia amygdalina* has protective and proliferative effects on VSMC at higher concentration and may inhibit the proliferation of cancer cells possibly through apoptosis. The anticancer property of the plant may be attributable to its high phenol content. We can conclude that *Vernonia amygdalina* extract has a good potential to be used as a new cancer therapeutic agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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