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Research Article

Medicinal Plant and Anticancer Activity on Neuroglioma

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ABSTRACT: This document file is a live template. The various components of your paper [title, text, heads, etc.] are exactly defined on the style sheet, as illustrated by the portions given in this document. Do not include any special characters, symbols, or math in your title or abstract. The authors must follow the guidelines given in the document for the papers to be published. You can use this document file as both an instruction set and as a template into which you can type your own text.

Key Words: **DHTR, sickle cell disease, anaemia, transfusion.**

1. INTRODUCTION

Neuroglioma is also known as gliocytoma and is a low grade brain tumor which arises in the cerebral hemisphere of the human nervous system. The World Health Organization identifies histological diagnosis as the international standard of care for brain tumors. However, the National Institutes of Health and American Cancer Society estimated neuroglioma affected more than twenty-three thousand people during the year 2016. Outpatient brain tumor treatments cost between ten or hundreds of thousands of US Dollars with insurance. As these costs exceed the income of most at risk patients, affordability of treatment may cause economic and financial hardship. However, clinical studies have shown natural medicinal herbal remedies removed cancer, and increased health. Therefore, to significantly reduce costs, medicinal natural products should be available to patients

The National Institute of Health has recorded sixteen chemotherapeutics approved for treatment of brain tumors by the United States Food and Drug Administration. The four 4 basic derivatives of approved chemotherapeutics for brain tumor treatment include everolimus,carmustine, bevacizumab, and temozolomide. Methods of administered doses include ingestible powders or capsules, intracranial or intravenous injections, and intracranial surgical implantation of slow release biofilms, patches, or wafers. Some common symptoms reported by patients before or during standard of care treatments included headaches, blurred vision, loss of hearing or appetite, light sensitivity, psychosis, depression, microbial infections, tachycardia, nausea, and vomiting. Moreover, surveys showed some antipsychotic medications and therapy assisted with patient recovery post-operation. Evaluations of diagnosis and patient monitoring are done by Magnetic Resonance Imaging, genetic sequences in circulated blood, biopsies, and histological examinations.

In humans, isocitrate dehydrogenase IDH occurs as three isozymes: Idh1, Idh2, and Idh3 which are encoded by five genes IDH1, IDH2, IDH3A, IDH3B, and IDH3G to convert isocitrate to alpha-ketoglutarate (a-KG) through oxidative Decarboxylation [1]. IDH1 and IDH2 use NADP+ electron acceptors to produce NADPH. Moreover, increase of NADPH by IDH1 reduced oxidative stress damage and lipid biosynthesis [2]. Furthermore, IDH1 was enhanced by hypoxic conditions in the catalysis of a-KG to isocitrate, which then is converted to acetyl-CoA for lipid metabolism. Moreover, short chain fatty acids enhanced blood brain barrier integrity with assembled tight junctions facilitated by intestinal epithelial membranes [3].Therefore, isocitrate dehydrogenase 1 (IDH1) as a hallmark of neurogliomas may mediate prevention and treatment of neurogliomas.

Vernonia amygdalina also known as bitter leaf, is a plant in East and West Africa. It is eaten raw or in soups and used with spices for its nutrition and to enhance health. Medicinal properties of *V. amygdalina* include flavinoids, phenolic compounds, free radical scavenging, metal ion chelation, and antioxidants [4]. Extracts with leaves of VA showed positive antibacterial activity with *Staphylococcus aureus* and *Pseudomonas aerginosa* [5]. Furthermore, water extraction VA leaves showed medicinal properties against triple negative breast cancer [6]. Moreover, VA enhanced pre-clinical treatments on tropical diseases, and a diversity of cancers. Upon treatment with VA on diabetes in Wistar rats, urinalysis showed differential glucose concentrations and enhanced liver function [7,8].

Air quality monitoring data during 2004-2013 in China showed redistribution of Polycyclic Aromatic Hydrocarbons PAHs in soil and was associated with increased cancer [9]. Fossil fuel mining, processing, and combustion releases carcinogenic carbon which increases effects of greenhouse gases and cancer incidence. Furthermore, mining, refinery facilities, incomplete combustable products, and processing wastes deposited near natural resources increased risk to health. During December 2015, Beijing declared a red alert because of health risks associated with haze, PAHs, and reduced air quality. Furthermore, economic losses impacted urban areas with closing of schools, roads, and factories.

The goal of this study was to determine half maximal lethal dose of VA on human neuroglioma H4 cells, measure oxidative stress via lipid per oxidation, and identify cell cycle arrest and apoptosis. Therefore bioassays included cell proliferation MTS, Lipid peroxidation malondialdihyde LPO-MDA, and Annexin V/Propidium Iodide with Flow Cytometry.

1. METHODOLOGY

2.1 Neuroglioma Cell Culture

Human neuroglioma brain tumor H4 HTB-148 cell line was purchased from ATCC. Dulbecco's Modified Eagle's Medium DMEM, 10% Fetal Bovine Serum FBS, 1% Penicillin/Streptomycin was the complete growth medium. Cells were seeded in T25 flasks and were incubated at 37C with 5% Carbon dioxide. The cultured cells were washed with 1X Phosphate Buffered Saline PBS Solution and fresh growth medium was added every two days.

A. *2.2 VA Treatment Sample Preparations*

Treatment preparation included stock and working VA extracts. VA stocks were prepared in dimethylsulfoxide and then deionized water was added to VA-DMSO to a total concentration of one thousand 1000 micrograms per one 1 milliliter. Extracts were pipetted into the six-well plates with confluent human neuroglioma H4 cells in different concentrations 0, 8, 16, 32 microgram per milliliters. After twenty-four hour incubations at 37C and 5% CO2, treatment samples were prepared according to manufacturers protocols for the following bioassays.

2.3 Cell Proliferation MTS Assay

Cell Proliferation CellTiter 96 Aqueous One Solution MTS bioassay was purchased from Promega. Confluent Human H4 neuroglioma cells were seeded into six-well plates. VA extracts were pipetted in different concentrations to 0, 8, 16, 32 microgram per milliliters. Treatment samples were incubated for 24, 48, 72, and 96 hours at 37C and 5% CO2. Treatment samples were transferred in 96 well plates and MTS pipetted 96well plate according to manufactured protocols. The prepared 96well plates were analyzed in a spectrophotometer microplate reader at optical density 490 nm wavelength. Experiments were performed in triplicate for statistical validity. Statistical analysis included means, standard deviations and ANOVA.

2.4 Malondialdehyde MDA Lipid Peroxidation LPO

Fluorimetric Lipid peroxidation LPO malondialdehyde MDA was purchased from Abcam. Human neuroglioma H4 cells were seeded into six-well plates. Upon confluence, prepared extracts were pipetted in different concentrations 0, 4, 8, 16, 32, 64 microgram per milliliters and incubated at 37C and 5% CO2. After incubation for twenty-four hours, samples were prepared into 96well plates by manufactured protocols. Treated samples were measured for MDA and standard curve was plotted by spectrophotometer microplate reader values at 490 nm wavelength. For statistical validity experiments were performed in triplicates. Methodology for statistical analysis included means, standard deviations and ANOVA.

2.5 Annexin V/ Propidium Iodine Flow Cytometry

Human neuroglioma H4 cells were seeded into six-well plates upon confluence of 1x10^6 cell proliferation prepared extracts were pipetted in different concentrations to 0, 4, 8, 16, 32, 64 microgram per milliliters. After twenty-four 24 hours of incubations at 37C and 5% CO2, samples were prepared according to manufacturers protocols.

2. RESULTS AND DISCUSSION

Upon treatment of human H4 neuroglioma HTB-148 with VA extracts, MTS cell proliferation bioassay showed cell viability was reduced. Furthermore, treatments with VA on neuroglioma showed increased concentration of malondialdehyde and increased lipid peroxidation in LPO-MDA assay. Upon treatments of human neuroglioma H4 cells with VA, annexin V and propidium iodide showed cell cycle arrest and apoptosis in Cellometer Flow Cytometry. Sequencing of tumor DNA circulated in patients plasma identified thirteen mutations which included TP53, IDH1 or TERT promoter [10]. Glial cell line-derived neurotrophic factors increased glioma cell proliferation with REC-alpha serine/threonine kinases (AKT) and c-Jun N-terminal kinase (JNK) [11]. Alkylation of DNA has shown toxicity to bone marrow [12]. Chinese medicinal plant herbal extracts suppressed cytokines TNF-a, IL-1B and IL-6 in hemeoxygenase deficient mice and neural inflammation was reduced [13].

Expression of occludin by brain endothelial cells was enhanced by intestinal gut microbiota, and lipid biosynthesis to blood-brain-barrier [14]. Decreased gut microbiota were associated with increased permeability in the blood brain barrier BBB and enhanced expression of the tight junctions. Selectivity of expressed transmembrane proteins across the BBB enhanced the cross-membrane transport and delivery to active sites in human neuroglioma. However, increased adenosine triphosphate ATP in human H4 neuroglioma cells increased alpha synuclein, and inhibited neurogenesis [15]. Intracellular protein expression of meriolins on arctic medicinal marine invertebrates inhibited cyclin-dependent kinase, decreased ATP [16]. Furthermore, medicinal plant extracts of *Magnolia officinalis* showed treatment of isolate honokiol on H4 cells increased p53, and apoptosis evaluated by Annexin V-PI and Flow cytometry [17]. Treatment with medicinal plant *Herbarium varium*, *Ocimicum hypargyrea*, and *Verticullus truncatula* isolated secondary metabolites, denatured and reduced cholinesterase, tyrosinase, a-amylase, and a- glucosidase [18].

Electrophilic natural products can form covalent bonds between to ligands at specific biologically reactive sites and inhibition by vernolide in VA increased oxidative stress to induce cytotoxicity [19]. MTT assays were used to measure cell viability in HepG-2 liver cancer cells [20]. All plant extract samples tested were less toxic to non-cancer normal

human keratinocytes HaCaT cell line [21]. Multi-resistant cancer cell lines showed overexposed ABC transporters, breast cancer resistance protein (BCRP), P-glycoprotein (P-gp), epidermal growth factor receptor (EGFR), and tumor suppressor mutations in p53 [22].

PAHs in soil samples during 2013 were 30.2%, 38.8%, 12.7%, and 18.3% from coal combustion, vehicle emissions, coking, and biomass burning respectively with reduced coal combustion since 2004 and increased vehicle emission PAHs [23]. Isolated DNA in a Chinese population with 72 glioblastoma patients and 320 healthy controls showed upregulated RTEL1 increased risk for developing malignant tumors [24]. Ionization and radiation induced apoptosis and increased proportionally with copies of TP53 and inversely correlated with cancer risk [25].

At a concentration of 50µg/mL, ethanolic extracts of propolis reduced cell proliferation on SF-295 human glioblastoma by 100% [26]. A mother in Brazil had an ultrasonography at 29 weeks of gestation which revealed microcephaly with calcifications in the fetal brain and placenta [27]. Studies with aqueous and ethanolic VA and leaf extracts showed transmission blocking activity of parasitic *P. bergei* and *An. stephensi* mosquitoes [28].

3. CONCLUSION

Upon treatment with VA on human H4 neuroglioma, MTS bioassay showed LD50 29 micrograms/mL per 1x10^6 cells. Furthermore, in the LPO-MDA bioassay, oxidative stress biomarker malondialdehyde measurements showed it increased. Cell cycle arrest and apoptosis was detected via propidium iodide and annexin V. Therefore, VA may be recommended for clinical trials and use in the United States in the treatment and prevention of cancers and neuroglioma.

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5. TABLES AND FIGURES

A. *Table 1. Cell Proliferation MTS Assay of Human Neuroglioma H4 Cells Treated with VA 96h*

8UG	F2	F3	F4	F5	F6	F7	F8	F9
	0.491	0.511	0.665	0.558	0.471	0.583	0.737	0.846
	0.492	0.513	0.667	0.558	0.472	0.584	0.738	0.848
	0.493	0.514	0.667	0.559	0.475	0.585	0.739	0.849
AVG 8ug/mL	0.492	0.513	0.666	0.558	0.473	0.584	0.738	0.848
16UG	F2	F3	F4	F5	F6	F7	F8	F9
	0.496	0.524	0.602	0.549	0.473	0.503	0.676	0.978
	0.494	0.526	0.604	0.55	0.474	0.505	0.677	0.979
	0.495	0.527	0.605	0.551	0.475	0.506	0.678	0.98
AVG 16ug/mL	0.495	0.526	0.604	0.55	0.474	0.507	0.677	0.979
32UG	F2	F3	F4	F5	F6	F7	F8	F9
	0.334	0.545	0.672	0.531	0.566	0.545	0.589	0.978
	0.334	0.547	0.673	0.531	0.566	0.546	0.59	0.978
	0.335	0.547	0.673	0.533	0.56	0.547	0.591	0.982
AVG 32ug/mL	0.334	0.546	0.673	0.532	0.564	0.546	0.59	0.979
Control	0.828							
	0.833							
	0.836							
Control AVG	0.832							

Figure 1. Lipidperoxidation MDA Assay of Human Neuroglioma H4 Cells Treated with VA 24h