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ANTICANCER POTENTIAL OF *VACCINIUM SECT.CYANOCOCCUS* AND *MOMORDICA CHARANTIA* MEDIATED HAFNIUM OXIDE NANOFORMULATION AGAINST ORAL CANCER CELL LINES – A COMPREHENSIVE STUDY

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ABSTRACT
The present study explored the anticancer activity of Hafnium Oxide nanoparticles incorporating *Vaccinium Sect.Cyanococcus and Momordica Charantia* against oral cancer cell lines (KB-1). Cell viability, AO/EtBr dual staining and ROS expression level using DCFH-DA staining were done to evaluate the impact of Hafnium oxide nanoparticles on oral cancer cells. The Hafnium Oxide nanoparticles demonstrated potent anticancer activity by inhibiting oral cancer cell proliferation and inducing cell cycle arrest in cancer cells. It was found that *Vaccinium Sect. Cyanococcus and Momordica Charantia* can stabilize the protein's function or restore it as a tumour suppressor.Also the hafnium oxide nanoparticles are showing wound healing activity in various concentrations at 24h.The Hafnium Oxide nanoformulation exhibited excellent cytotoxicity and potent anticancer effects, making it a potential candidate for non-toxic chemotherapy in cancer nanomedicine. Furthermore, the nanoparticles ability of wound healing activity highlights the effectiveness of hafnium oxide nanoparticles in the healing process of chronic wounds. This research contributes to the growing interest in Hafnium Oxide nanoparticles and their potential applications in cancer treatment.

KEYWORDS: *Vaccinium Sect.Cyanococcus* and *Momordica Charantia*, Nano formulation, Oral cancer, Inhibition of cell proliferation,Apoptosis

INTRODUCTION

Head and neck squamous cell carcinoma is the sixth leading cancer worldwide.At the same time, Oral Squamous Cell Carcinoma (OSCC) is the most commonly occurring malignancy among head and neck malignancies, and finding effective treatment and patient management has always been a significant priority.1 In 2020, Oral cancer was the 11th most common malignancy in men and the 18th most common malignancy in women worldwide. Globally, oral cancer accounted for approximately 377,000 new cancer cases in 2020, with a mortality rate of almost 50%, according to data given by the Global Cancer.2

Observatory (GCO).Timely detection and proper evaluation are significant as the treatment approaches, outcomes, and patient survival vary significant. Oral squamous cell carcinoma treatment includes surgery, radiation therapy, chemotherapy, targeted therapy, and immunotherapy.3Abundant clinical trials have been conducted to improve treatment efficacy, unmask genetic markers for a complete cure, and increase oral cancer patients' disease-free survival rate. Chemotherapy was the most frequently used (36.8%), while targeted therapy and immunotherapy each constituted 15.2% of the treatment strategies. Patients with advanced or recurrent Oral squamous cell carcinoma (OSCC) are usually treated with surgery, radiotherapy and chemotherapy.4Recently, targeted therapies, particularly Ferroptosis,have gained significant attention as potential treatment strategies. Nanoparticles-loaded natural products and their adjuvants can induce redox reactions and immunogenic effects in oral cancer cells and suppress immunosuppressive mechanisms in the tumour microenvironment.5

One of the most promising nanotechnology applications is in medicine, and nanoparticles can target particular cells in the body and deliver drugs directly to the disease site.This approach can potentially reduce side effects and improve the efficacy of treatments for cancer and other diseases.6 Polymeric nanoparticles possess a high level of chemical reactivity and can carry a substantial amount of drugs on their surface due to their small size. However, their small size makes them vulnerable to deposition and oxidation, which can result in losing their desired properties.Stabilizing agents like polymers, surfactants, and polysaccharides safeguard these nanoparticles.7
The effectiveness of these nanoparticles as catalysts and their potential for cytotoxicity are influenced by factors such as the type of polymer used, as well as the size and shape of the nanoparticles.8One effective method of stabilization involves encapsulating them within nanogels.

The study "Polymeric Nanoparticles as Promising Tool for Anticancer Therapeutics" delves into the application of polymeric nanoparticles in cancer therapies, offering valuable insights into their potential role in combating cancer.9
The modification of natural products is a crucial area for the efficient generation of highly potent molecules.*Vaccinium* is a common and widespread genus of shrubs in the heath family (Ericaceae). The genus of *Vaccinium Sect.Cyanococcus* species was first described by Carl Linneaus.10The name *Vaccinium* was used in classical latin for a plant, possibly the bilberry or a hyacinth, and may be derived from the Latin bacca, berry, although its ultimate derivation is obscure.11The taxonomy of the genus is complex and still under investigation. Genetic analysis indicates that the genus *Vaccinium* is not Monophyletic.Other health benefits of *Vaccinium* include presence of phytonutrients namely Vitamins A and Vitamin C which also render them an antioxidant property of protection of cells against disease free radicals. 12This antioxidant property of the *Vaccinium* inhibit tumor growth, decrease inflammation and may help to slow down the other types of cancers such as esophageal, lung, mouth, pancreatic and colon cancers.The antimicrobial activity of *vaccinium* has been proven due to the flavonoid fraction especially presence of anthocyanins also the anti-inflammatory activity of Vaccinium is having good response against lipopolysaccharide macrophages.With the benefits of all these, *Vaccinium sect. cyanococcus* has been employed in the present study.13

Momordica charantia (commonly called bitter melon, goya, bitter apple, bitter gourd, bitter squash, balsam-pear and many more names listed below) is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit.Its many varieties differ substantially in the shape and bitterness of the fruit through various postulated mechanisms. 14However, clinical trial data with human subjects are limited and flawed by poor study design and low statistical power.

Due to the presence of many bioactive compounds, some of which possess potent biological actions, this plant is used in folk medicine all over the world for the treatment of different pathologies, mainly diabetes, but also cancer, and other inflammation-associated diseases.15 However, the majority of existing studies on *M.charantia* bioactive compounds were performed only on cell lines and in animal models. Therefore, because the real impact of bitter melon on human health has not been thoroughly demonstrated, systematic clinical studies are needed to establish its efficacy and safety in patients.16

Hafnium is known as the “little brother” of titanium and zirconium. It has a large band gap (Eg > 5 eV), a high dielectric constant (r = 25), a high material density (9.6 g/cm3, a high melting point (over 2700 C) and chemical inertness,good dielectric properties and high chemical stability.17It can exist in three polymorphic structures such as the monoclinic (P21/c) at low temperature, the tetragonal (P42/nmc) at around 2000 K and the orthorhombic Pnma at about 2870 K. The unique properties of hafnium is its excellent corrosion resistance in aggressive environments and a very large neutron absorption cross section.Other biomedical applications of Hafnium oxide nanoparticles include anti-inflammatory and antioxidant activities.By impeding DNA damage and attenuating various metabolic pathways,these nanoparticles reduce inflammation.18

Silver nanoparticles can be used in a variety of goods, including bandages, gauzes, sutures, plasters, and many more lotions and ointments for wound healing.19Along with antibacterial capabilities, silver-treated textile materials and surgical sutures display better wound healing properties in vitro, demonstrating that silver has a favorable influence on cell migration and proliferation.However, silver nanoparticles are prone to aggregation, which may result in a change in size and a loss of antibacterial activity. As a result, the creation of materials with evenly dispersed silver in the polymer matrix is a hot area for future study.20

In the present study, we explored the anticancer activity of *Vaccinium Sect.Cyanococcus* and *Momordica Charantia* mediated nanoformulation synthesized using Hafnium Oxide as a stabilizing agent in oral cancer cell lines. We also aimed to assess the effectiveness of Hf NPs in the healing process of wounds using cell lines.

MATERIAL AND METHODS

1.Synthesis and Characterization of *Vaccinium and Momordica* Mediated Hafnium Oxide Nanoparticles

Fresh *Momordica charantia* (bitter melon) and *Vaccinium sect. Cyanococcus* (blueberry)fruits were purchased from a nearby market. After being cleaned and allowed to dry in the shade, the plant materials were ground into a fine powder. 10 g of powdered plant material was boiled for 30 minutes in 100 mL of distilled water to create aqueous extracts, which were then filtered through Whatman No. 1 filter paper. *Vaccinium and Momordica* mediated hafnium oxide nanoparticles (HfO_2 NPs) were formed by adding a solution of hafnium chloride ($HfCl_4$) dropwise to the plant extract while stirring continuously at room temperature.After centrifuging and drying at 60°C, the nanoparticles were cleaned with distilled water.The concentrations used in biological assays were selected based on preliminary dose response pilot experiments.UV-Analysis was done to confirm the synthesis of nanoformulation.

2.Cell line maintenance

Oral cancer cell lines (KB-1) were obtained from the NCCS, Pune. The cells were grown in T25 culture flasks containing Dulbecco's Modified Eagle Medium supplemented with 10% FBS and 1% antibiotics. Cells were then maintained at 37◦C in a humidified atmosphere containing 5% CO2. Upon reaching confluency, the cells were trypsinized and passaged.

3.Cell viability (MTT) assay

The cell viability of 4 different formulations of *Vaccinium Sect.Cyanococcus* and *Momordica Charantia* mediated hafnium oxide nanoformulation treated with oral cancer (KB-1) cells was assessed by MTT assay. The assay is based on the reducing capacity of metabolically active cells to convert soluble yellow tetrazolium salt to insoluble purple formazan crystals. KB-1 cells were plated in 96 well plates at 5 × 103 cells/well concentrations. The cells were washed twice with 100 µl of serum-free medium 24 h after plating, and the cells were made to starve by incubating them in serum-free medium at 37ºC for 3 h.Then, cells were treated with different concentrations of nanoformulation for 24h and 48 h. At the end of treatment, the medium from control and nanoformulation treated cells were discarded, and 100 µl of MTT containing DMEM (0.5 mg/ml) was added to each well. The cells were then incubated at 37ºC in the CO2 incubator for four hours.The MTT medium was discarded, and the cells were washed with 1×PBS.Then, the formazan crystals formed were dissolved in dimethyl sulfoxide (100 µl) and incubated in the dark for one hour.The developed colour intensity was assayed using a Micro ELISA plate reader at 570 nm.The viable cell count was expressed as a percentage of control cells cultured in a serum-free medium..Cell viability in the control without any treatment was considered 100%.The cell viability was calculated using the formula: Percentage cell viability=[A570 nm of treated cells/A570 nm of control cells]×100 with different concentrations of nanoformulation for 24h and 48 h.At the end of treatment, the medium from control and *Vaccinium and Momordica* hafnium nanoformulation treated cells were discarded, and 100 µl of MTT containing DMEM (0.5 mg/ml) was added to each well. The cells were then incubated at 37ºC in the CO2 incubator for four hours.The MTT medium was discarded, and the cells were washed with 1×PBS. Then, the formazan crystals formed were dissolved in dimethyl sulfoxide (100 µl) and incubated in the dark for one hour.

4.Morphology study

Based on the MTT assay, we selected the optimal doses, 5 and 10 µl/ml (IC-50:10.29 µl/ml) for further studies. A Phase contrast microscope analyzed morphologic changes of cells. 2× 105 cells were seeded in 6 well plates and treated with 5 µl/ml and 10 µl/ml and 20 µl/ml of *Vaccinium and Momordica* Hafnium oxide nanoformulation for 24 h.The medium was removed at the end of the incubation period, and cells were washed once with a phosphate buffer saline (PBS) at pH 7.4. The plates were then observed under a phase contrast microscope.

5.ROS expression level using DCFH-DA staining.

The intracellular ROS level in treated KB-1 cells was analyzed by DCFDA staining. The cells were grown in 24 well plates and treated with nanoformulation (5 and 10 µl/ml) for 24 h time points. After the nanoformulation treatment, the cells were incubated with 200 µl of DCFDA (10 µM) working solution at 37 °C for 20 min. After incubation, the DCFH-DA working solution was removed, and cells were washed with PBS and the intracellular ROS level under the fluorescence microscope.

6.Determination of mode of cell death by acridine orange (AO)/ethidium bromide (EtBr) dual staining

The effects of Vaccinium and Momordica hafnium nanoformulation in KB-1 (5 and 10 µl/ml) cell death were also determined by AO/EtBr dual staining as described previously. The cells were treated with *Vaccinium and Momordica* hafnium nanoformulation (10 µl/ml) for 24 h, harvested, and washed with ice-cold PBS. The pellets were resuspended in 5 µl of acridine orange (1 mg/mL) and 5 µl of EtBr (1 mg/mL). The stained cells apoptotic changes were then observed using a fluorescence microscope.

7.Determination of Wound Healing Activity

Wound scratch assay

The scratch wound healing assay is a widely employed and versatile in vitro technique for assessing cell migration and proliferation under diverse experimental conditions, including gene knockdown and chemical treatments. In this method, a "wound" or gap is created in a confluent monolayer of cells using a sterile pipette tip, and the subsequent cell migration and growth into the gap are monitored over time to evaluate wound closure. This assay is simple, cost-effective, and easily customizable, with the potential for high-throughput adaptation using automated systems. In the present study, Oral cancer cells namely KB-Cells India, were cultured in high-glucose supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution. Cells were maintained at 37 °C in a CO2 incubator with 5% CO2 and 18–20% O2. Passage 15 cells were seeded at a density of 0.25 million per well in a 6-well plate and cultured to 80–100% confluence. A cross-shaped scratch was then carefully made using a sterile 200 µL pipette tip without changing the medium. Detached cells were removed by gentle washing, and the remaining cells were treated with the desired test compounds and incubated for an additional 48 h. Images of the wound area were captured at various time points (0 and 24h) using consistent microscope settings.Wound closure was quantitatively analyzed using ImageJ software, with multiple fields per well documented to reduce variability, and all experiments were conducted in replicates to ensure reliability.

8.Statistical analysis

All data obtained were analyzed by One way ANOVA flowed by Students-t-test using SPSS, represented as mean±SD for triplicates. The level of statistical significance was set at $p<0.05$.

RESULT

1.UV spectroscopy

The preliminary and quantitative analysis confirmed using a UV visible absorption spectrum, the obtained multiple absorption peaks of *Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated Hafnium oxide nanoparticles as shown in Fig. 1. The absorption peaks at~350 nm. (in the presence of (C–C) bonds), 400 nm representing hybridization and 500 nm corresponding to interaction between the intermolecular interactions.

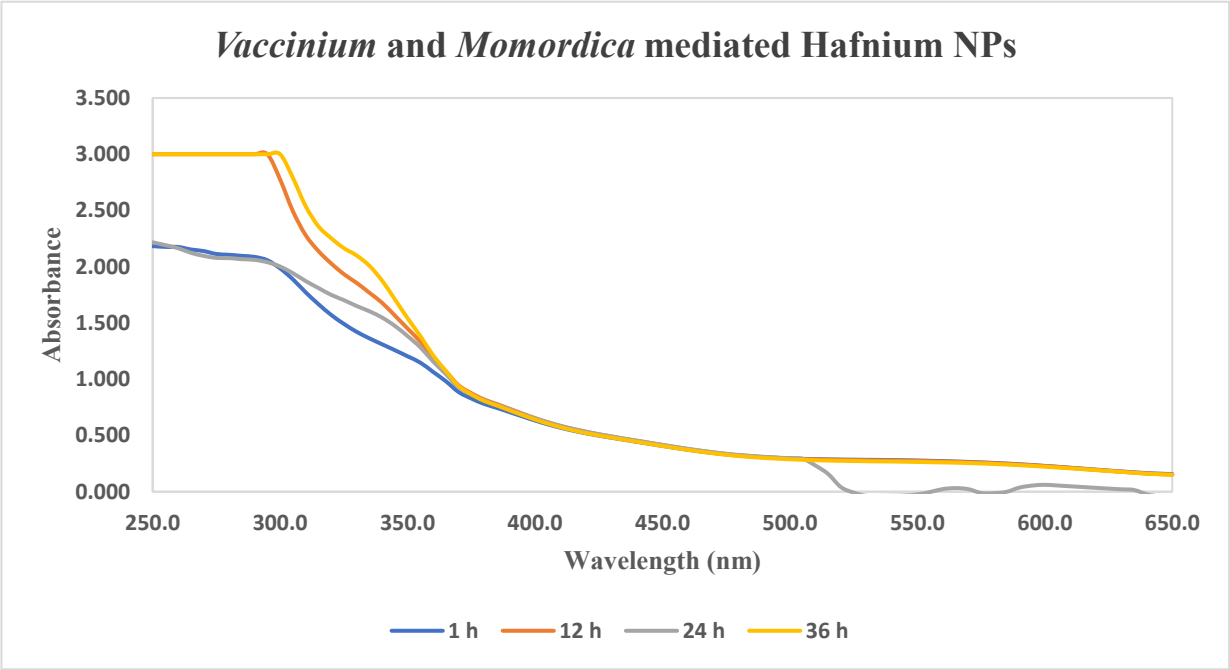


Fig.1 UV-visible spectroscopy of *Vaccinium Sect.Cyanococcus and Momordica Charantia* hafnium

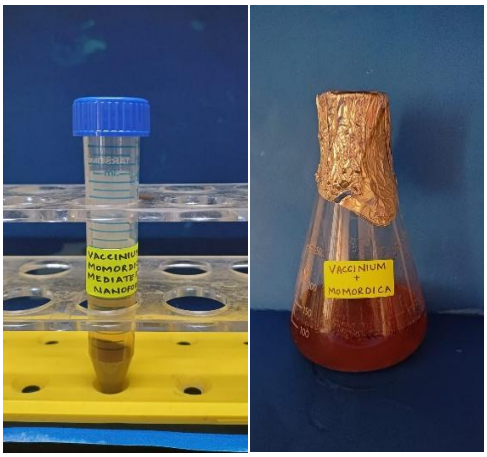


Fig 2. *Vaccinium and Momordica* mediated Hafnium NF

2.Effect of *Vaccinium Sect. Cyanococcus and Momordica Charantia* mediated Hafnium oxide nanoformulation on Cell viability and Cell Morphological Changes of Oral Cancer Cell Line

The MTT assay assessed the anticancer activity of the formulations of *Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated Hafnium Oxide nanoparticles against oral cancer cells. The nanoformulations exhibited a significant dose-dependent reduction in cell viability compared to the control group ($p<0.05$) when treated with formulations, but the nanoformulation with the highest dosage of *Vaccinium Sect. Cyanococcus and Momordica Charantia* showed the maximum cytotoxicity.The morphological changes observed in oral cancer cells (KB-1) after treatment with various nanoformulation concentrations.Phase-contrast microscopy revealed noticeable alterations, including apoptotic bodies, cell shrinkage, and detachment of cells.The nanoformulation treatments administered 24 h in oral cancer cells (KB-1) resulted in substantial and dose-dependent cytotoxicity (with a p-value less than 0.001). Invitro cytotoxicity of the

nanoformulation of varying concentrations was investigated using an MTT assay on the KB cell line for 24 h.Live cells reduced the MTT and the resultant formazan is directly proportional to the cell viability. Results showed that the viability of cancer cells was inhibited in a dose-dependent manner with a 50% inhibitory concentration (IC50) of 13.42 µM. The addition of 25 µM of ICN-K04 decreased the % cell viability to 23.7%.

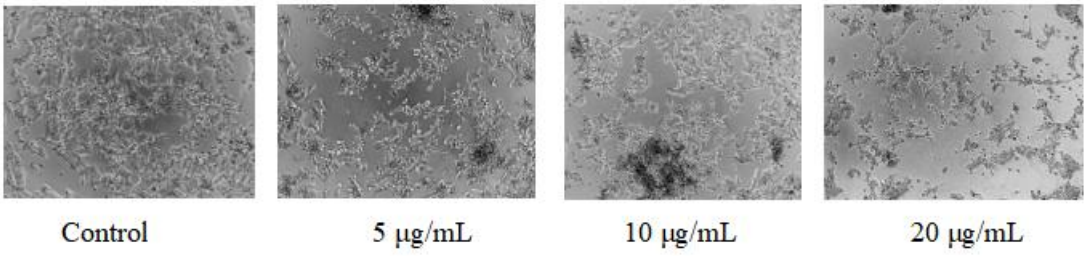


Fig.3 Morphological changes in Oral cancer cells at various concentrations treated with *Vaccinium and Momordica* mediated Hafnium Oxide Nanoparticles

3.*Vaccinium Sect. Cyanococcus and Momordica Charantia* mediated nanoformulation induces apoptosis in oral cancer cells

In this study, we investigated the cytotoxic effects on cancer cells and identified an excessive expression of ROS as a potential cause for the observed cytotoxicity. To explore this further, they exposed oral cancer cells (KB-1) to two concentrations of nanoformulations, specifically 5 and 10 µg/mL, for 24 h. The level of ROS expression was evaluated using DCFH-DA staining, and the results indicated that both nanoformulation concentrations led to a significant increase in ROS expression in the oral cancer cells (KB-1). Notably, the cells treated with 10 µl/ml exhibited a higher intensity of green fluorescence than those treated with 5 µl/mL of nanoformulation. Our present investigation utilized the AO/EtBr staining method to evaluate apoptosis-mediated cell death in nanoformulation treated cells. This approach allowed us to assess cell nuclei morphology in untreated control cells and those exposed to nanoformulation.

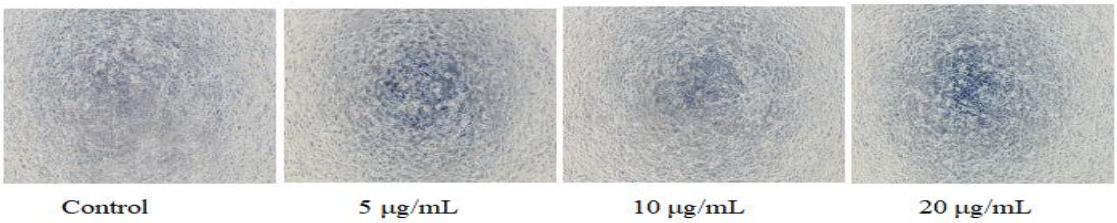


Fig.4.Cell Viability and Biocompatibility in Oral cancer cells at various concentrations treated with *Vaccinium and Momordica* mediated Hafnium Oxide Nanoparticles

During the staining process, live cells displayed a consistent green colour, early apoptotic cells exhibited a yellow stain, and late apoptotic cells presented condensed and often fragmented nuclei with an incorporated ethidium bromide stain appearing red. The AO/EtBr staining method corroborated our quantitative apoptosis analysis results, providing further evidence that the highest rate of cell death, characterized by nuclear deformation and loss of cell wall integrity, was explicitly observed in KB-1 cancer cells treated with nanoformulation.To determine the occurrence of apoptosis in cultured oral cancer cells, we examined both untreated and *Vaccinium Sect. Cyanococcus* and *Momordica Charantia* mediated Hafnium oxide nanoformulations treated KB-1 cells using Annexin-V and PI. Compared to the untreated group,*Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated Hafnium Oxide nanoparticles treated KB-1 cells displayed a higher percentage of apoptotic cells. Specifically, the percentage of apoptotic cells significantly increased in *Vaccinium and Momordica* hafnium oxide treated KB-1 cells (38.33%, n=3, $P \leq 0.05$) compared to untreated cells (14.63%). No necrotic signs were observed in *Vaccinium Sect. Cyanococcus* and *Momordica* mediated Hafnium oxide treated cell lines, while control cells exhibited only 0.33% necrosis.

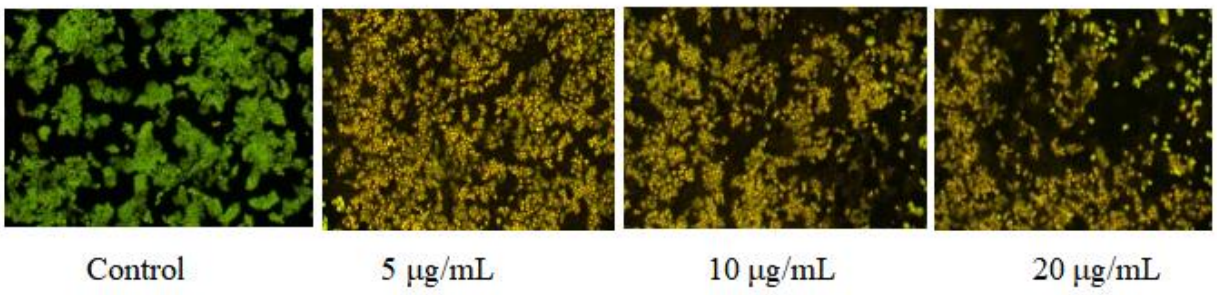


Fig.5. AO/EtBr staining in Oral cancer cells at various concentrations treated with *Vaccinium and Momordica* mediated Hafnium Oxide Nanoparticles

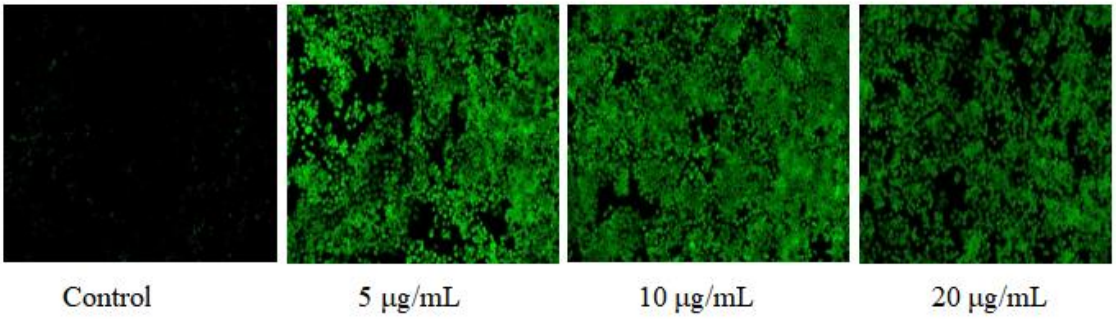
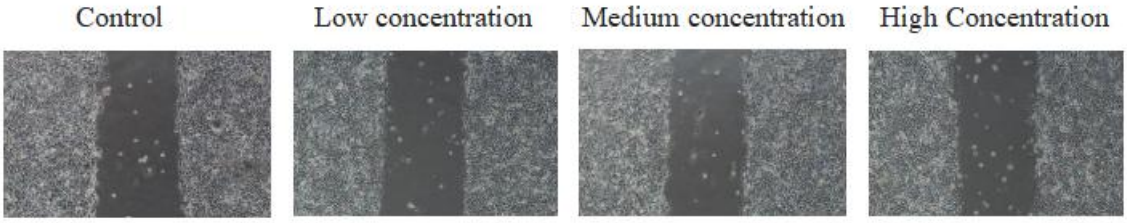


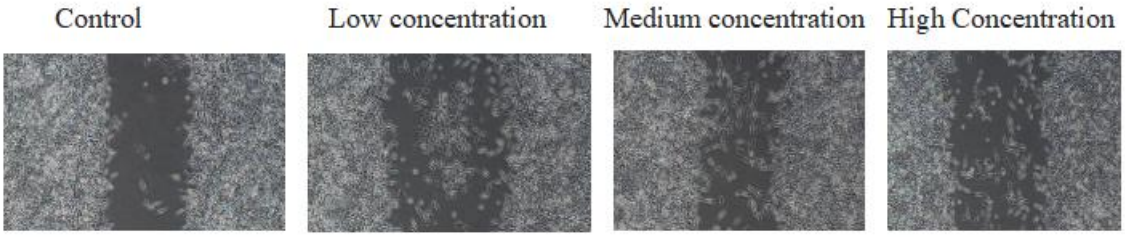
Fig.6. DCFH-DA staining in Oral cancer cells at various concentrations treated with *Vaccinium and Momordica* mediated Hafnium Oxide Nanoparticles

4.Determination of Wound Healing Activity of *Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated Hafnium Oxide Nanoformulation

The scratch test revealed that *Vaccinium Sect.Cyanococcus and Momordica Charantia* HfNPs dose-dependently increased the rate of wound closure. After 24 h exposure, the HfNPs dose-dependently increased the rate of wound closure; nonetheless, a significant increase was observed at concentrations of 15 and 20 µg/mL, compared to the control group. After 24 h exposure, the HfNPs dose-dependently increased the rate of wound closure; however, a significant increase was detected at a higher concentrations.



At 0h concentration



At 24h concentration *Vaccinium Sect.Cyanococcus and Momordica Charantia* Hafnium Oxide Nanoparticles group

Fig.7.Scratch wound assay in normal cells at various concentrations treated with *Vaccinium and Momordica* mediated Hafnium Oxide Nanoparticles

DISCUSSION

The results demonstrate that the *Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated nanoformulation exerts potent anticancer effects against oral cancer cells.21 MTT assay is one of most popular and reliable viability assays for evaluating anticancer activity of synthetic and natural compounds, which depend on the conversion of the substrate to chromogenic products by the action of mitochondrial reductase in live cells,22which involves the conversion of water-soluble yellow dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to an insoluble purple formazan.23In the present study, we used four different formulations of *Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated nanoformulation, namely a control group low,medium and high concentration using MTT assay.24There was a dose-dependent inhibition in the cell viability of KB-1 cells after 24 h for all the four formulations of nanoformulation, in which *Vaccinium Sect.Cyanococcus* and *Momordica Charantia* mediated nanoformulation showed the maximum cytotoxicity against oral cancer cells.25The nanoformulation inhibited cell viability and induced apoptosis dose-dependently with a 50% inhibitory concentration of 10.29 µl/ml. IC-50 at 10.29 µl/ml shows the effectiveness of *Vaccinium Sect.Cyanococcus and Momordica Charantia* against oral cancer cells even at lesser concentrations.26Sheela DL et al. studied the viability of human hepatocellular carcinoma,colon cancer cells and murine macrophages using MTT assay.27They found dose-dependent cytotoxicity towards all three cell lines by LA from 0 to 80 mg/mL after 48 h. In another study28, the cytotoxicity of Virgin, Processed, and fractionated coconut oil in different concentrations was investigated for 72 h using MTT and reported a prominent growth inhibition against the KB cell line by 20% of processed coconut oil and 5% of fractioned coconut oil.29 The antiproliferative activity of LA was further emphasized by Lappano

et al. from the study against Ishikawa endometrial cancer cells and SkBr3 breast cells. The results showed significant growth inhibition of the tumour cells without affecting the normal breast epithelial cells (MCF-10A).30Antitumour activity of *Vaccinium Sect.Cyanococcus and Momordica Charantia* against squamous cell carcinoma, colon, kidney, leukemia, myeloma and breast cancer cells was rreported the cytotoxic effects of fungal-derived silver nanoparticles in various concentrations from 1.75 to 50 µl/ml on oral cancer cell lines (SCC-9) using an MTT assay.31They found a 50% inhibitory concentration at 12 µl/ml. Wimardhani et al50 compared the effects of cisplatin and low molecular weight chitosan on Ca9-22 oral cancer cells using MTT assay and found a decline in the viability of Ca9-22 cells with IC50 800±131.45 µg/mL (LMWC) and 8±0.029 µg/mL (cisplatin).32The IC-50 concentration of *Vaccinium Sect.Cyanococcus* and *Momordica Charantia* was fixed, and the quantification of apoptosis induction was per formed using flow cytometry.33

The major clinical challenge in treating the cancer is the relapse prevalence, which occurs due to the failure of the primary treatment regimen in targeting the cancerous cells.34Therefore, drugs having the potential of huge accessibility and specifically delivered to the target site hold a great promise in the management of cancer.35Nanoparticle-based drug delivery system has gained huge attention due to its versatile approach to accessing the cancerous site and another inflammatory milieu.36Thus, the synthesized nanoparticles have a unique potential to overcome the poor penetrating ability and also exert their maximum efficacy to control the proliferation of the disease.37

To determine the functional impact of ALEM on the metastatic potential of the SCC-15 cell line, a wound healing assay was performed in the present research work as the progression of a tumor is not only reliant on the pace at which it proliferates.38The research findings demonstrated that ALEM and ALEM-AuNPs demonstrated their potential to stop the migration of cancer cells in the wound healing experiment by delaying the growth of treated SCC-15 cells towards the center of the wound area when compared to the control.39In the present study, the HfNPs dose-dependently increased the rate of wound closure; however, a significant increase was detected at a higher concentrations.40

While there was an observable rise in apoptotic cells in the presence of *Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated nanoformulation, the percentage of apoptotic cells was notably higher in treated *Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated nanoformulation41 Early and late apoptotic cells: 38.33%.A study reported the excellent antioxidant and anti-inflammatory properties of Chitosan thiocolchicoside lauric acid nanogel in oxidative stress-related disorders and inflammatory conditions in an in vitro analysis.42 Overall, the present study provides compelling evidence of the potential of the *Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated nanoformulation as a promising therapeutic candidate for oral cancer treatment.43The nanoformulation's ability to induce apoptosis and disrupt vital molecular pathways in cancer progression makes it a potential non-toxic chemotherapy option with added antibacterial properties.44Further research and in vivo studies are warranted to validate its efficacy and safety for clinical translation in cancer nanomedicine.45

In patients with oral cancers, the application of radiation therapy causes severe side effects, including progressive fibrosis of blood vessels or hypocellularity eventually leading to complications in bone healing and difficult rehabilitation (Yerit et al., 2006; Nelson et al., 2007).46 Hu et al. (2018) reported that copper-doped borate bioactive glass/poly (lactic-co-glycolic acid) dressing loaded with vitamin E stimulates endothelial cell migration, proliferation, and fibroblast cell proliferation. Another study conducted by Alizadeh (2019) demonstrated that a 1 mM concentration of 80 nm CuNPs accelerated wound healing over a shorter time via the formation of granulation tissue and higher new blood vessels.Nitric oxide (NO) plays a critical role in the regulation of various wound-healing processes, including inflammatory response, cell proliferation, collagen formation, and angiogenesis.47

All the rounded cells indicate the induction of the apoptotic pathway, and many reports have mentioned these alterations as morphologic hallmarks of apoptosis.48 Diverse types of integrin compounds are known to have a predominant role in the crosstalk between cell and matrix. These processes were performed through focal adhesion and linked kinases.49Once the trigger happens for the initiation of apoptosis, all kinds of focal adhesion and linked kinases will be deactivated. All these prove that the synthesized nanoparticles have the potential to induce cell death in oral cancer cells by initiating the process of apoptosis.50To further validate the process of apoptosis, the experiments were performed with AO/EB dual staining. The concept behind this staining is a clear indication to understand the exact impact of apoptosis in cancer cells.51 AO tends to integrate into both the normal and early apoptotic cells where the cell membrane is intact. This results in the formation of a fluorescent green color after linking to the DNA. Moreover, EtBr stain has the property to attach only to the damaged cells and this reaction gives the orange-red color fluorescence when attached to the DNA.52

So, the present study established a simple, eco-friendly, and economical method to generate therapeutically valuable HfNPs using *Vaccinium and Momordica*, which is predominantly available in India.53Oral cancer is a significant health concern globally, and any research exploring potential treatments for this condition is highly relevant and valuable.The study utilized an innovative method by synthesizing HfNPs from the *Vaccinium and Momordica* plant extract.54

Promising anticancer properties showed a novel approach to potential cancer treatment.Using plant extracts to synthesize nanoparticles, implies a natural and potentially eco-friendly method for developing therapeutic agents, which could be advantageous for future drug development.55

In addition, ROS analysis was performed to understand the mechanism of apoptosis. Mostly, all the cellular-based ROS tend to trigger the cell cycle arrest and eventually induce apoptosis in cancer cells.56To note, even reports suggest that ROS can arrest the metastatic process.The chemotherapy process for cancer treatment mostly targets the ROS mechanism resulting in the induction of apoptosis. In our study, the synthesized nanoparticle tended to show a promising rise in the ROS in a dose dependent manner.All these reports and data demonstrate that the nanoparticles induce apoptosis by altering the ROS levels. Few reports have discussed the importance of green synthesis and characterization and evaluated the biological activity of *S. trilobatum*-mediated AgNPs.In a recent study55, researchers tried to utilize the *S. trilobatum* fruit extract in various dimensions. They evaluated the anticancer activity of the fruit extract in breast cancer cell lines. We could not find any studies in the literature on the anti-cancer activity of the leaf extracts of this plant in oral cancer cell lines.56

In cancer therapy, tumor growth can be suppressed by activating the apoptotic machinery in the cell.Many malignant cells, however, are unable to regulate the genes that control apoptosis, rendering them resistant to the induction of apoptosis by a variety of stimuli, including intracellular and extracellular signals chemotherapeutic drugs, and radiotherapy.57A previous study has investigated that the SH-SY5Y cells exposed to rotenone caused about 50% cell death, whereas isolongifolene pre-treated cells dose-dependently regulated the toxic effects of rotenone by which it exhibits neuroprotective effect against apoptosis. In our study, we investigated the cytotoxicity of nanoformulation at different cell lines for 24 h.The result exhibited that the viability of cancer cells was inhibited by 50% in a dose-dependent manner, and also we found that 50% inhibitory concentration (IC50) of at 13.42 μM among all other concentrations respectively.57

CONCLUSION

In this research, a nanoformulation containing *Vaccinium and Momordica Charantia* was prepared using hafnium oxide as a mediator and was successfully synthesized and characterized using UV-vis spectroscopy, and the nanoformulation demonstrated promising anticancer properties against oral cancer cells.Additionally, the hafnium-based *Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated nanoformulation induced oxidative stress in the cancer cells, as indicated by increased levels of ROS. Based on the results, we concluded that this *Vaccinium Sect.Cyanococcus and Momordica Charantia* mediated nanoformulation , has the potential as a cytotoxic agent against oral cancer cells.The presence of a nanoparticle coating with various functional groups presents opportunities for further modifications to enhance its biological activities, including antimicrobial and anticancer effects, while also improving biocompatibility.

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