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Herbal Formulation Synthesis Of Hafnium Oxide Nanoparticles And Its Anti-Inflammatory,Anti-Oxidant And Cytotoxic Effects

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Abstract:
Objective*Herbal formulations offer an intriguing option for managing various health concerns, including designed hypertension. This study investigated Green sythesised Hafnium Oxide Nanoparticles, which includes extracts of Vaccinium Sect.Cyanococcus and Momordica Charantia.*
Methods *Additionally, Anti-Inflammatory potential of the Hafnium Oxide nanoparticles was analyzed using Egg albumin denaturation assay and Bovine serum Albumin assay.The study screened in vitro antioxidant activity using Hydrogen Peroxide assay.The Nanoformulation was evaluated for cytotoxic effect using brine shrimp lethality assay and was compared with the commercial.*
Result *The results demonstrated that Hafnium Oxide nanoparticles exhibited the highest antioxidant activity.The Herbal formulation mediated synthesis of Hafnium oxide nanoparticles showed some positive outcomes both in anti-oxidant and anti-inflammatory activities when compared to commercial nanoformulation.*
Conclusion *Herbal mediated HfONPs might contribute to show potential as antimicrobial agents in future dentistry.*
Keywords *: Herbal formulation,Nanoparticles,Hafnium oxide,Green synthesis.*

INTRODUCTION

Nanotechnology is an emerging field of interest for many researchers to develop newer drugs for commercial use.It plays a vital role in managing various diseases cancer disease,heart disease and various other diagnostic and therapeutics techniques Odyemi S et al (2015).The medical implementations of nanobiotechnology has ushered in a new era of nanobiomedicine by introducing the idea of controlling and treating the biological systems of humans using nanomaterials Wilhelmia et al(2020).

Over a period of time the researchers had developed a huge attention of unique features of metal oxide nanoparticles such as supermagnetic behaviour,selective catalytic activity,sensitivity and other optical properties Gheorghe et al(2023).Since Hafnium oxide nanoparticles (HfONPs) are generally recognized as safe and less toxic, they are frequently chosen over other metal oxide nanoparticles.HfONPs possess several biomedical applications such as antibacterial activity, anti-inflammatory activity, antidiabetic activity, and anti-cancer effect(In a recent research work, the functionalised Hafnium oxide nanoparticles (HfONPs) have shown acting as a radioenhancer showing both anti-cancer effect towards the the soft tissue sarcomas and other cancerous cells Jia s et al (2018).

The 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 Yang et al (2016).This 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 Chiu et al.The antimicrobial activity of Vaccinium has been proven due to the flavonoid fraction especially presence of anthocyanins also the antiinflammatory 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 Pereira et al (2019).

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.Abundant pre-clinical studies have documented the anti-diabetic and hypoglycaemic effects of M.charantia through various postulated mechanisms Chiu et al and Pereira et al (2019).

The current study involves the green synthesis of HfONPs with Vaccinium and Momordica formulation. To evaluate their antimicrobial activity, an agar well diffusion technique was utilized to test against oral pathogens compared to standard.Furthermore, we used the brine shrimp lethality test to evaluate the cytotoxicity effect of the prepared HfONPs-based herbal nanoformulation.There is a growing interest in herbal products due to their potential to offer fewer side effects than conventional antihypertensive medications. Herbal therapies, rooted in ancient practices, are increasingly recognized for their potential in managing chronic conditions like HTN Selvapriya et al (2021).Although natural products remain underexplored in contemporary research, they are extensively used in drug discovery and development.Combining herbal formulations with complementary properties can significantly enhance therapeutic outcomes, particularly when targeting multiple mechanisms of blood pressure regulation. Synergistic pharmacological effects from combined herbal formulations are increasingly acknowledged for their potential to improve therapeutic efficacy against chronic disorders. Using two or more herbs often yields better results than relying on a single herb, especially when their components are synergized. Thus, incorporating multiple plants with similar biological activities may enhance therapeutic efficacy against serious conditions.Monika et al.

Materials and methods

1.Plant materials and extraction

Vaccinium and Momordica leaf powder were collected from a commercial store. One gram of Vaccinium powder and one gram of Momordica powder were added to 100 mL of distilled water. The mixture was heated in a mantle for 15-20 minutes at 40-50°C. After heating, the solution was cooled and filtered using Whatman No. 1 filter paper (Whatman Plc, Maidstone, UK). The resulting formulation was then used for further studies.

2.Preparation of HfONPs

A total of 30 mM of Hafnium Oxide was dissolved in 50 mL of distilled water to make a zinc nitrate solution. Subsequently, 50 mL of the Vaccinium Sect. Cyanococcus and Momordica Charantia formulation was added to the prepared Hafnium oxide solution.The resulting mixture solution was placed in a magnetic stirrer set at 600-700 rpm (rotations per minute).Nanoparticle synthesis was analyzed using a UV-visible double-beam spectrophotometer.After 24 hours,the solution was removed from the stirrer to record readings and observe color changes.The solution gradually darkened over time compared to its initial color. The nanoparticles were then centrifuged at 8,000 rpm for 10 minutes.

3.Anti-inflammatory Activity of Herbal formulation mediated hafnium oxide nanoparticles:

Egg Albumin Denaturation Assay :

The anti-inflammatory activity of the herbal nanoformulation was determined. The samples used for this assay include 0.2 mL of egg albumin (fresh), 2.8 mL of phosphate-buffered saline (PBS) at pH 6.4, and 0.6 mL of these hafnium oxide nanoparticles at various concentrations dissolved in 0.2% DMSO. The concentrations of the hafnium oxide nanoparticles in the total reaction solution ranged from 10-50 µg/mL. The samples were incubated for 10 minutes at 37°C and then heated at 70°C in a water bath for an additional 20 minutes to induce denaturation of the egg albumin. After cooling the mixture, the absorbance was measured at 660 nm. Negative controls consisting of 0.2 mL of fresh egg albumin, 0.6 mL of 0.2% DMSO, and 2.8 mL of PBS were included in the experiment. Diclofenac sodium was used as a positive control for the study.

Bovine Serum Albumin Assay

The anti-inflammatory activity of the herbal nanoformulation was evaluated.To assess the anti-inflammatory activity, 0.05 mL of the Hafnium oxide nanoparticles were taken, and various concentrations ranging from 10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL, and 50µg/mL were added to 0.45 mL of a 1% aqueous solution of bovine serum albumin. The pH of the solution was corrected to 6.3 using a small amount of 1N hydrochloric acid. These samples

were then incubated at room temperature for 20 minutes, followed by heating at 55°C for 30 minutes in a water bath. After the heating process, the samples were allowed to cool down, and the absorbance was measured using a spectrophotometer at 660 nm. Diclofenac sodium was the standard drug for comparison. Dimethyl sulfoxide (DMSO) was used as a control in this experiment. The percentage of protein denaturation was determined using the following
equation: % Inhibition = (Absorbance of control -Absorbance of sample/Absorbance of control) x 100.

4.Anti- oxidant Activity of Herbal Formulation mediated Hafnium Oxide Nanoparticles

Hydrogen Peroxide Assay :

Overall, 1 mL of reaction mixture with 100 mL of 28 mM of 2-deoxy-2-ribose was prepared. To that various concentrations of the green synthesised hafnium oxide nanoparticles (10-50 µg/mL) were added. Along with that, 200 mL of ferric chloride, 200 µL of ethylenediaminetetraacetic acid, and 100 mL of ascorbic acid were mixed.Then, it was incubated for an hour at 37°C and the OD was measured at the wavelength of 532 nm against the blank solution. Tocopherol was chosen as a control. The following formula was used: hydroxyl radical scavenging activity (%) = ((A blank - A sample)/Ablank) × 100, where A blank is the absorbance of the control reaction (without sample), and A sample is the absorbance of the reaction with the sample.

5.Cytotoxic Activity of Hafnium Oxide Nanoparticles

Cytotoxic effect using brine shrimp lethality assay In 200 mL of distilled water, 2 g of iodine-free salt was added and mixed thoroughly. A six-well ELISA (enzyme-linked immunosorbent assay) plate reader was taken, and 10-12 mL of saline water was added carefully. Subsequently, 10 nauplii were slowly added to each well. The prepared HfONPs-based mouthwash was then added to each well at different concentrations (5 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL).The sixth well was used as a control (containing only salt water and live nauplii, without any samples) as noted in previous studies.A comparison was made between the HfONPs-based nanoformulation.The ELISA plate was left for incubation for
approximately 24 hours. After incubation, the plates were analyzed and the number of live nauplii present was noted down. The percentage of live nauplii was estimated using the following formula: (Number of dead nauplii/Number of dead nauplii + Number of live nauplii) × 100.

RESULT

The anti-inflammatory activity of these Herbal Nanoformulation were evaluated using the bovine serum albumin denaturation assay and egg albumin denaturation assay. These nanoparticles were tested at different concentrations, and their inhibitory effects were compared to standard values. The results for the egg albumin denaturation assay showed a
percentage inhibition at a various concentration of 30% at 10 µg/mL, 45% at 20 µg/mL, 35 % at 30 µg/mL, 85% at 40 µg/mL, and 40% at 50 µg/mL whereas the results of Bovine serum albumin denaturation assay revealed a percentage of inhibition at a various concentration of 10% at 10 µg/mL, 50% at 20 µg/mL, 40% at 30 µg/mL, 35% at 40 µg/mL, and 60% at 50 µg/mL.

These values indicated that these nanoparticles exhibited significant anti-inflammatory activity by inhibiting both bovine serum albumin denaturation and egg albumin denaturation assay.Moreover, the anti-inflammatory properties of these hafnium oxide nanoparticles were comparable to the standard diclofenac sodium at all tested concentrations.The antioxidant activity of these green synthesised hafnium oxide nanoparticles was also assessed using Hydrogen peroxide assay and it was found that there was percentage inhibition of 65 % at a concentration of 50 µg/ml.

The cytotoxic effects of Herbal mediated nanoformulation were evaluated using the brine shrimp lethality assay. This assay is commonly employed to assess the cytotoxicity of substances by measuring their impact on the survival of the brine shrimp nauplii. In the present study, a control group without any drug was maintained to establish a baseline for calculating the percentage of live nauplii. The results of the cytotoxicity assessment indicated that different concentrations of the nanoformulation exhibited varying effects on nauplii survival.At a concentration of 5 µg/mL Herbal mediated nanoformulation, approximately

50% of the nauplii remained alive.Similarly, at concentrations of 20 μg/mL and 40 μg/mL, the nanoformulation resulted in the preservation of approximately 60% of live nauplii. However,
at a higher concentration of 80 μg/mL, only 55% of the nauplii survived.The control group comprising of plant extract resulted in preservation of all the 100% nauplii.

Graph 1.Anti-inflammatory activity of Herbal Nanoformulation synthesised using Vaccinium Sect.Cyanococcus and Momordica Charantia

Graph 2.Anti-Oxidant activity of Herbal Nanoformulation synthesised using Vaccinium Sect.Cyanococcus and Momordica Charantia

Graph 3. Cytotoxic Activity of Herbal Nanoformulation synthesised using Vaccinium Sect.Cyanococcus and Momordica Charantia

DISCUSSION

A detailed investigation of the various uses of HfNPs mediated by herbal formulations from Vaccinium Sect.Cyanococcus and Momordica Charantia explains their potential in the
thrombolytic, antibacterial, and antioxidant domains Yang et al and Chiu et al.These biogenic HfNPs are effective radical scavengers, as shown by their antioxidant effectiveness
in DPPH and H2O2 experiments.Their activity was compared with that of ascorbic acid, a well established standard for antioxidant measurements.7This demonstrates the potential of these nanoparticles to reduce oxidative stress, and suggests a possible direction for the development of antioxidant treatments.These results highlight the natural antioxidant qualities of plant formulations and the increased effectiveness of nanoparticle mediation, recommending further research into their potential medicinal uses Selvapriya et al (2021).The antioxidant efficacy of Vaccinium Sect.Cyanococcus and Momordica Charantia formulation-mediated HfNPs was assessed using DPPH and H2O2 assay, revealing a dose-dependent enhancement in antioxidant activity Yang et al.Similarly, in the present various uses of HfONPs mediated by herbal formulations from Vaccinium.Sect Cyanococcus and Momordica Charantia explained their potential in the Anti-oxidant and Anti-inflammatory properties.The Anti-oxidant property was 65% at a concentration of 50 μg/ml which was slightly lower when compared to anti-oxidant property of 76% in HfNPs mediated by herbal formulations from Vaccinium Sect.Cyanococcus and Momordica Charantia. In a study(Liang et al (2016), the anti-inflammatory effect of herbal formulation prepared from Vaccinium Sect. Cyanococcus and Momordica Charantia was studied with different concentrations like 10 μL, 20 μL, 30 μL, 40 μL and 50 μL. 50 μL was the highest concentration showing more percentage of inhibition of 91.5%, which demonstrates good anti-inflammatory properties due to the synergistic action of the herbal formulation whereas the anti-inflammatory property of herbal formulation mediated HfONPs was found to be 85% at 40μL.These results prove that in the present study,the herbal synthesised HfONPs showed favourable Anti-inflammatory and Anti-oxidant properties.Hence,further research is needed to broaden their potential medicinal uses.

In another study,Chen et al (2016), the cytotoxic activity of herbal mouthwash formulation was assessed by using Brine shrimp Lethality test.The mouthwash formulation did not show cytotoxic activity on brine shrimp as all the shrimps in the 6 wells in which the extract were added survived on the first day. From the brine shrimp lethality test done, it was noted that on
the first day all the nauplii survived, and on the second day 8 nauplii survived at lower concentrations(Sun et al).

As far as the concentration of the herbal mouthwash formulation, the cytotoxicity was found to be better at lower concentrations where 8 nauplii survived.In the present study,the cytotoxic activity of herbal formulation mediated HfONPs was 50-60% at all concentrations which means the nanoformulation showed less cytotoxic activity on brine shrimp.In the second day,5-6 nauplii survived in all concentrations Jain et al (2019).

Author contributions

Dr.Rajesh Kumar.S:Conceptualization, Formal analysis, Visualization, Funding acquisition, Supervision, Writing–original draft, Writing–review & editing.

Dr.Surabhi Halder:Conceptualization, Investigation, Methodology, Visualization, Data curation, Writing–original draft, Writing–review & editing, Investigation, Methodology, Visualization, Data curation, Writing–original draft.Haejoon Park: Investigation, Methodology.

Conflicts of interest

"There are no conflicts to declare".

Data availability

The Data were recorded and statistically significant values were generated using SPSS software.

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REFERENCES

1.Odeyemi S, Afolayan A, Bradely G. In vitro anti-inflammatory and free radical scavenging activities of crude saponins extracted from Albuca bracteata Jacq. Bulb. Afr J Tradit Complement Altern Med 2015;12:34-40.

2.Wilhelmina Kalt,Aedin Cassidy, Luke R Howard, Robert Krikorian, April J Stull, Francois Tremblay and Raul Zamora-Ros.Recent Research on the Health Benefits of
Blueberries and Their Anthocyanins.Adv Nutr.2020 Mar; 11(2): 224–236.

3.Gheorghe Adrian Martău, Teleky Bernadette-Emőke, Răzvan Odocheanu,Dacian Andrei Soporan, Mihai Bochiş,Elemer Simon and Dan Cristian Vodnar.Vaccinium Species (Ericaceae): Phytochemistry and Biological Properties of Medicinal Plants.Molecules.2023 Feb; 28(4): 1533.

4.Jia S, Shen M, Zhang F, Xie J. Recent Advances in Momordica charantia: Functional Components and BiologicalActivities. Int J Mol Sci. 2017 Nov 28;18(12):2555.

5.Yang D, Liang XC, Shi Y, Sun Q, Liu D, Liu W, et al. Anti-oxidative and anti-inflammatory effects of cinnamaldehyde on protecting high glucose-induced damage in cultured dorsal root ganglion neurons of rats. Chin J Integr Med 2016;22:19-27.

6.Chiu YJ, Huang TH, Chiu CS, Lu TC, Chen YW, Peng WH, et al. Analgesic and antiinflammatory activities of the aqueous extract from Plectranthus amboinicus (Lour.) Spreng. Both in vitro and in vivo.

7.Pereira WD, Geetha RV, Thangavelu L (2019) Anti-inflammatory activity of Punicagranatum extract on oral microbes - In vitro. International Journal of Research in Pharmaceutical Sciences 10:1019–1022.

8.Selvapriya, S., Priyadharshini, R., Rajeshkumar, S. and Sinduja, P. (2021) "Anti-Inflammatory and Cytotoxic Effect of Stevia and Neem Based Herbal Formulation", Journal of Pharmaceutical Research International, 33(59A), pp. 150–160.

9.Monica K, Rajeshkumar S, Ramasubramanian A, Ramani P, Sukumaran G. Anti-inflammatory and antimicrobial effects of herbal formulation using karpooravalli, mint, and cinnamon on wound pathogens.