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RESEARCH ARTICLE

ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITY OF HAFNIUM OXIDE NANOPARTICLES SYNTHESISED USING MOMORDICA CHARANTIA

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Abstract

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Green synthesised Hafnium oxide nanoparticles can be prepared for various other applications to address oral health concerns like bacterial infections and inflammation.The current study explored the anti-inflammatory and antioxidant activity of the synthesized Hafnium Oxide Nanoparticles.

Materials and Methods

Hafnium Oxide nanoparticles were prepared using Momordica Charantia plant extract and tested for potential applications.The anti-inflammatory activity of the produced nanoformulation was examined using a bovine serum albumin denaturation assay, egg albumin denaturation assay and membrane stabilization assay whereas the antioxidant activity of these HfONPs was assessed using DPPH and Hydrogen peroxide assays.Structural and compositional characterisation of these HfONPs involved UV-visible spectroscopy and Scanning Electron Microscopy (SEM).

Results

The prepared Momordica Charantia mediated Hafnium oxide Nanoparticles possesses significant anti-inflammatory activity.The Momordica Charantia extracts mediated by Hafnium oxide nanoparticles at 50µl concentration showed the maximum anti-inflammatory activity and antioxidant activity.

Conclusion

This study successfully synthesized Hafnium oxide nanoparticles using Momordica Charantia and thoroughly characterized their structural and compositional attributes.These green synthesised hafnium oxide nanoparticles using Momordica Charantia showed anti-inflammatory and antioxidant activity suggesting its potential for a very good anti-cancer effect and other inflammatory conditions.

Key Words : Hafnium Oxide,Nanoparticles,Green synthesised,Oral health,Bacterial infections.

Background

Introduction

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.[1]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.[2]However, 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.[3]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.[4]Besides, both *in vitro* and *in vivo* studies have demonstrated that bitter melon may also elicit toxic or adverse effects under different conditions.[5]The aim of this paper is to provide an overview of anti-inflammatory and antioxidant properties of bitter melon, discussing its pharmacological activity as well as the potential adverse effects.The fruits and leaves of *Momordica* species are rich in phytochemicals and may have many health-promoting effects by offering nutritional and nutraceutical components. The plant has been known for ages and it has been used in many traditional and folk medicines for a wide range of medical applications including the treatment of Type-2 Diabetes mellitus, hypertension, obesity, cancer, bacterial and viral infections.[6]

Hafnium (Hf) 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.[7]

It 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.[8]

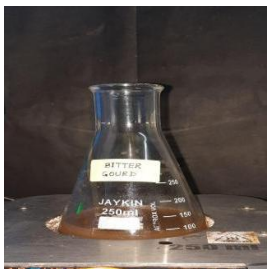
The green synthesis method, which uses plants and microorganisms, has emerged as the best technique for producing nanoparticles.[9]Green synthesis for creating nanoparticles is considered safer and more environmentally friendly because of its potential for stabilizing and reducing any potential damage to the environment.Green synthesis or the environment friendly synthesis refers to the development of processes and methodologies.[10]It minimizes the use of harmful substances and reduce the production of waste.It was validated by Pal et al[10] in another study that green synthesis has gained significant attention in various field of pharmaceutical industry.When it comes to green synthesis, researchers find alternatives with environment friendly routes to produce pharmaceutical compounds.[11]

This research paper describes the successful synthesis of *Momordica Charantia* mediated Hafnium oxide Nanoparticles using green synthesis method.These functionalized Hafnium Oxide nanoparticles were characterized using the most common characterisation techniques.In order to broaden their biomedical application, we aimed to demonstrate the anti-inflammatory and anti-oxidant properties of these green synthesized nanoparticles.

Materials and Methods

1.Synthesis of Plant extract

Powdered *Momordica Charantia* was used in the present study.Accurately 1g of each powder was taken and then added to the 100 mL distilled water.It was then boiled at 70°C for around 30 minutes. The boiling helps in activating the phytochemicals present in the extract.



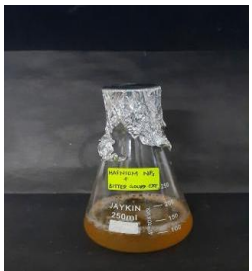


Figure:1.Preparation of nanoparticles using *Momordica Charantia* plant extract

(a) Powder was weighed (b) Boiling of the Plant extract (c) Hafnium oxide nanoparticles

2.Biosynthesis of Hafnium Oxide Nanoparticles.

One molar solution of the extract was created by thoroughly mixing the mixture, and it was heated at 60 degrees Celsius for 15 to 20 minutes and filtered. The filtered *Momordica Charantia* extracts were mixed with 0.016 g of Hafnium Chloride and 90 ml of distilled water and then alternately stirred and shaken at 900 rpm using a magnetic stirrer and an orbitol shaker. Periodically, an observation was made to assess the color change of the solution, and it was photographed and recorded.Throughout this period, the progress of the reaction was routinely monitored by measuring the UV-visible spectra at specific time intervals up to the full 24-hour duration.

3.Anti- oxidant Activity of green synthesised hafnium oxide nanoparticles.

(a) 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.

(a) DPPH Assay :

2,2-Diphenyl-1-Picryl Hydrazyl (DPPH) Free Radical Scavenging Assay The antioxidant activity of the green synthesised hafnium oxide was analyzed using the DPPH assay. Various concentrations (10µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL) of the nanogel were mixed with 1 mL of DPPH (0.1 mM) in methanol and 450 µg/mL of 50 mM Tris-HCl buffer at pH 7.4. The mixture was then incubated in a dark room for 30 minutes. The reduction in the quantity of the DPPH free radical was assessed by measuring the absorbance at 517 nm.This measurement indicated the antioxidant capacity of these hafnium oxide nanoparticles.Ascorbic acid was used as standard in this assay. By evaluating the absorbance at 517 nm, this assay determined the antioxidant activity of the green synthesised hafnium oxide nanoparticles. The percentage of the inhibition was determined from the following equation: % inhibition of sample = (Absorbance of control - Absorbance of sample/Absorbance of control) x 100.

4.Anti-inflammatory Activity of green synthesised hafnium oxide nanoparticles :

(a) Egg Albumin Denaturation Assay :

The anti-inflammatory activity of the green synthesised hafnium oxide nanoparticles 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.

(b) Bovine Serum Albumin Assay

The anti-inflammatory activity of the green synthesised hafnium oxide nanoparticles was evaluated as described by Das et al. 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.

(c) Membrane stabilisation assay :

Fresh human blood was collected in a sterile tube containing anticoagulants. The blood was centrifuged for 10 minutes at 1000 g at room temperature to separate the RBCs from other blood components. The supernatant was slowly removed and the RBCs left behind were washed three times using PBS. Then RBCs were resuspended in Tris-HCl buffer to obtain a 10% (v/v) RBC suspension.

1mL of the RBC suspension was pipetted into each centrifuge tube and different concentrations of these green synthesised hafnium oxide nanoparticles were added to each tube, gently mixed, and incubated for 30 minutes at 37°C. The centrifuge tubes were then centrifuged at 1000 g for 10 minutes at room temperature to pellet the RBCs. The absorbance of the supernatant obtained was measured at 540 nm using an ultraviolet spectrophotometer.

The percentage inhibition of hemolysis was calculated using the following formula: % Inhibition = {(OD control - OD sample)/OD control} x 100. OD control in the formula is the absorbance of the RBC suspension without the test compound and OD sample is the absorbance of the RBC suspension with the test compound.

5.Characterization of green synthesized Hafnium oxide nanoparticles

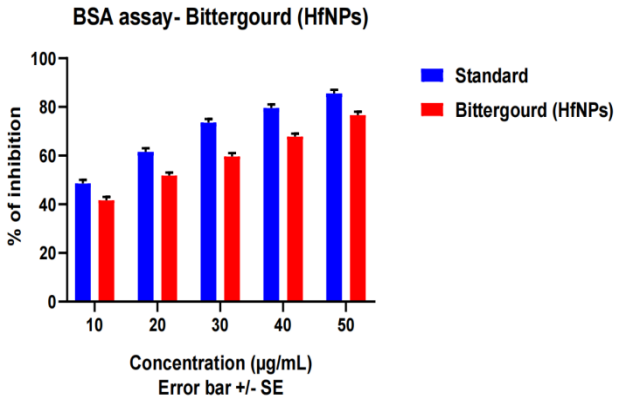
For the morphological analysis, scanning electron microscopy (SEM) (JEOL FE SEM IT-800, [JEOL Ltd., Tokyo, Japan]) was employed. The absorption characteristics of the synthesized nanoparticles were ascertained via the utilization of a double-beam UV-visible spectrophotometer (ESICO - model 3375 [Electronics India Ltd., Panchkula, India] encompassing a wavelength range spanning from 350 nm to 550 nm.

Statistical analysis

The values were tabulated in Microsoft Excel (Microsoft Corporation, Redmond, WA) and transferred to SPSS Statistics version 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) for statistical analysis. An independent t-test was carried out between the control and experimental groups at 10 µl, 20 µl, 30 µl, 40 µl, and 50 µl concentrations. Any p-value less than 0.05 was considered significant.

Result

The anti-inflammatory activity of these green synthesized hafnium oxide nanoparticles 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 bovine serum albumin denaturation assay showed a percentage inhibition at a various concentration of 43% at 10 µg/mL,50% at 20 µg/mL, 60% at 30 µg/mL, 65% at 40 µg/mL, and 70% at 50 µg/mL whereas the results of egg albumin denaturation assay revealed a percentage of inhibition at a various concentration of 50% at 10 µg/mL, 55% at 20 µg/mL,60% at 30 µg/mL, 65% at 40 µg/mL, and 70% 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.Also the anti-inflammatory activity of these green synthesised hafnium oxide nanoparticles was assessed using the human red blood cell membrane stabilization assay. The nanoparticles were tested at different concentrations and compared to standard values. The results revealed a percentage inhibition of 50% at a concentration of 10 µg/mL, 60% at 20 µg/mL, 65% at 30 µg/mL, 70% at 40 µg/mL, and 82% at 50 µg/mL against 60%, 65%, 70%, 75% and 85% for the standard, diclofenac sodium at same concentrations.These findings showed a significant anti-inflammatory activity of these nanoparticles in the membrane stabilization assay.



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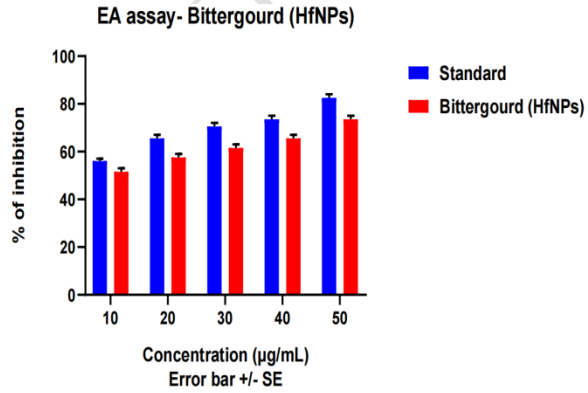

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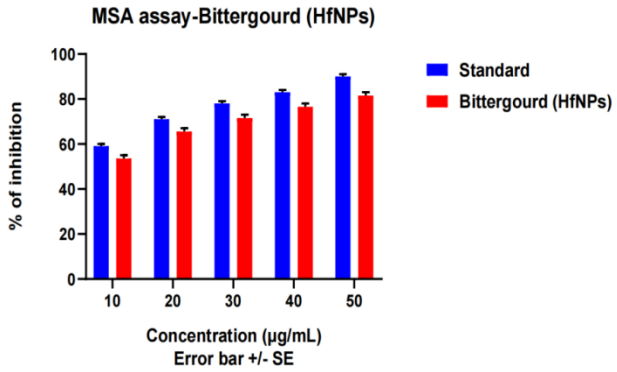
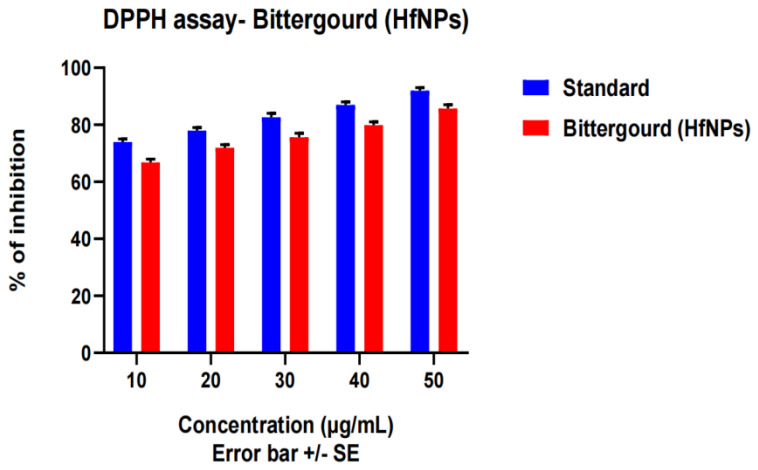


(c)

Figure:2.Estimation of Anti-inflammatory activity of *Momordica Charantia* synthesized Hafnium oxide Nanoparticles using (a) Bovine serum Albumin assay, (b) Egg Albumin assay, (c) Membrane stabilisation assay

The antioxidant activity was done by using the DPPH method. DPPH assay was compared from a lower concentration to a higher concentration of these green sythesised hafnium oxide nanoparticles. In these green synthesised hafnium oxide nanoparticles, a different concentration were added. In the different concentrations of these green synthesised hafnium oxide nanoparticles, 10µg/ml had a 65% inhibition, 20 µg/ml had a 70% inhibition, 30 µg/ml had a 75% inhibition, 40 µg/ml had a 80% of inhibition and 50 µg/ml had a 85% of inhibition.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.



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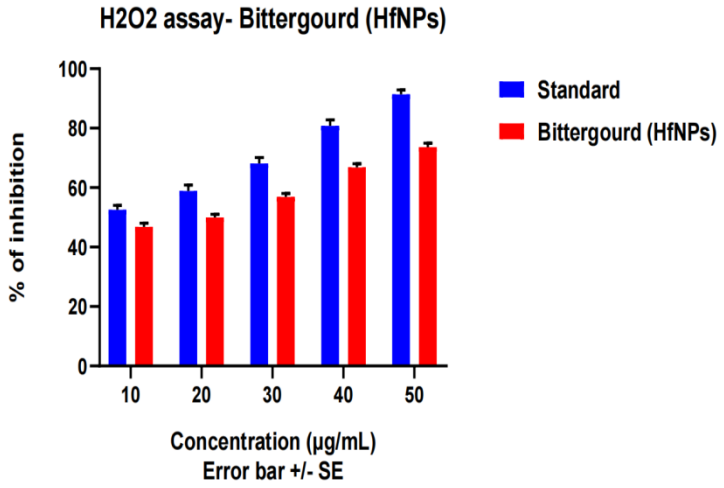

(b)

Figure:3.Estimation of Antioxidant activity of *Momordica Charantia* synthesized Hafnium oxide Nanoparticles using (a) DPPH assay , (b) Hydrogen peroxide assay

Visual observation

The synthesis of Hafnium oxide nanoparticles mediated by *Momordica Charantia* extract was successfully carried out.The initial image of the Hafnium oxide nanoparticles showed a yellow colour, indicating the presence of synthesized nanoparticles.

UV-visible spectrophotometry

UV-visible spectroscopy was performed to characterize the optical properties of *Momordica Charantia extract* mediated HfONPs.The analysis revealed a prominent absorption peak at a wavelength of 480 nm.The UV-visible spectrum displayed a strong absorption band centered at 440 nm, indicating the presence of HfONPs.The absorption peak corresponds to the electronic transitions within the HfO nanoparticles, specifically involving the excitation of electrons from the valence band to the conduction band.The observed absorption peak is consistent with the literature-reported optical properties of HfONPs.The location and intensity of the absorption peak provide valuable insights into the size, morphology, and composition of the synthesized HfONPs. The absorption maximum at 460 nm suggests the formation of small-sized HfONPs, as larger particles tend to exhibit absorption peaks at longer wavelengths.The efficient synthesis of HfONPs using the *Momordica Charantia* mediated approach is evident from the distinct absorption peak observed at 480 nm. The choice of *Momordica Charantia* as a reducing and capping agent has proven successful in producing stable and well-defined HfONPs with desirable optical properties. In conclusion, the UV-visible spectroscopy analysis of *Momordica Charantia* mediated HfONPs revealed a maximum absorption peak at 480 nm, indicating the successful synthesis of HfONPs. These findings support the potential use of *Momordica Charantia* as a promising natural source for the fabrication of HfONPs with distinct optical properties.

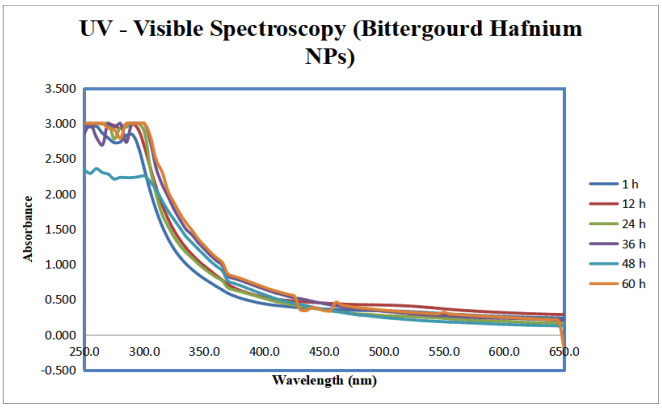

Figure:4.Uv-visible spectroscopy of *Vaccinium sect. Cyanococcus* synthesized Hafnium oxide Nanoparticles

Scanning electron microscope (SEM)

The scanning electron microscopy (SEM) analysis of *Momordica Charantia* mediated HfONPs revealed their morphological characteristics, which can be seen in Figure. The nanoparticles exhibited a spherical shape and were observed to be aggregated. The size of the nanoparticles was determined to be approximately 100 nm. The SEM image showed that the HfONPs synthesized using *Momordica Charantia* displayed a uniform spherical morphology.The nanoparticles appeared to be well-dispersed, forming aggregates of varying sizes.The aggregation of nanoparticles is a common phenomenon during the synthesis process, and it can be attributed to various factors such as van der Waals, electrostatic interactions, and solvent evaporation effect.The size determination was based on the analysis of multiple nanoparticles from the SEM image. It is important to note that the observed size may slightly vary due to the aggregation of nanoparticles, leading to overlapping or partial obscuration of individual particles.

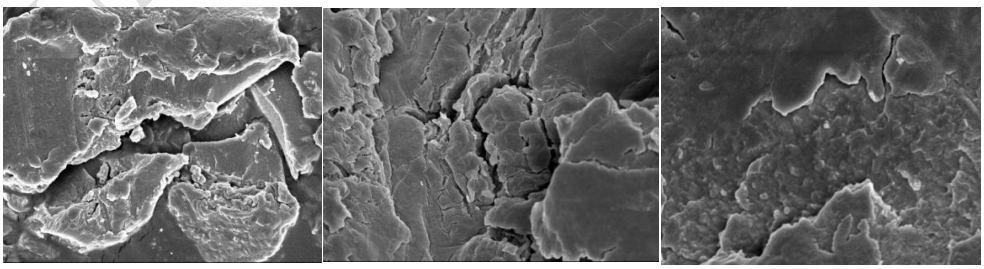

Figure:5.SEM images of Momordica Charantia synthesized Hafnium oxide Nanoparticles

Discussion

The present study aimed to evaluate the antioxidant and anti-inflammatory potentials of Hafnium oxide nanoparticle-mediated *Momordica Charantia* combination through in vitro assays.[11]The findings of this study provide valuable insights into the therapeutic implications of the nanoformulation and its potential as a natural remedy for conditions associated with oxidative stress and inflammation.[12]The anti-inflammatory and antioxidant properties of the formulation were assessed it was found that these HfONPs showed better properties at lower concentrations and at higher values, its properties were comparable with the respective controls.*Momordica Charantia* also commonly known as Bitter Gourd contain many primary antioxidants and nutrients.[13]They contain various phytonutrients namely lycopene, lignans, carotenoids, and reasonable amounts of vitamin A, zeaxanthin, and lutein.All these phytonutrients help in fighting free radicals that are produced in our body as a result of body metabolism.In the present study we synthesized Bittergourd mediated hafnium oxide nanoparticles using green synthesis method of preparation.[14,15]

However in a study[16], the color of the zinc oxide, chamomile, and green tea combination leaf extract solution mixture changed from yellowish to brownish-black during the green synthesis of zinc oxide nanoparticles.[17]In the present study, there was no change in color throughout the reaction.The Uv-vis spectroscopy throughout the reaction confirmed the synthesis of HfONPs having showing the absorption peak of 480 nm.

The antioxidant property of *Momordica charantia* is due to presence of flavonoids and other polyphenols.They also fight against the inflammation in the body as they are packed with polyphenols.[18]In the present study, the anti-oxidant and anti-inflammatory activities of these bittergourd synthesized hafnium oxide nanoparticles had been briefly described.The antioxidant activity of these bitter gourd synthesized HfONPs were studied using DPPH and Hydrogen peroxide assays.It was found that the maximum percentage inhibition by these HfONPs were found to be 85% at 50 µg/mL in DPPH assay and a percentage inhibition of 65% at a concentration of 50 µg/mL was found in Hydrogen peroxide assay.The anti-inflammatory

activity of these synthesized HfONPs were studied briefly using Bovine serum denaturation assay, egg albumin denaturation assay and membrane stabilization assay.The findings indicate that the *Momordica charantia* synthesized HfONPs have a very promising anti-oxidant and anti-inflammatory effect.[19]In a study conducted by Shubhangini et al[20], the antioxidant and anti-inflammatory activity of CTLA Nanogel was found to be comparitively less than the results of the present study.

Conclusion

Within the limits of the present study, it can be concluded that the anti-inflammatory and antioxidant properties of *Momordica charantia* mediated hafnium oxide nanoparticles were found to be higher at The presence of flavonoids and polyphenols in bittergourd gives them a very good antioxidant property.In the present study, the bittergourd mediated synthesis of hafnium oxide nanoparticles shows a promising anti-microbial activity predominantly in *C.Albican* species compared other oral pathogens.

These findings suggests that these green synthesized hafnium oxide nanoparticles have potential anticancer property against precancerous lesions of the oral cavity such as candidal infections.Prosthetic rehabilitation of most of the acquired maxillary defects of head and neck requires careful supervision of the site.The green synthesized hafnium oxide nanoparticles in the present study with exceptional antimicrobial,antioxidant and anti-inflammatory activity can be used as an adjuvant therapy after the surgical resection at the defect site before the definitive treatment is advised at various follow up period throughout the treatment.Further investigation on these green synthesised HfONPs should be carried in future studies against the fibroblast cell lines to confirm the effectiveness of these HfONPs.

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