FishTaxa - Journal of Fish Taxonomy
Vol 38, ISSN: 2458-942X

Embryonic toxicology evaluation of Hafnium oxide nanoparticles synthesized using Momordica charantia and Vaccinium sect.cyanococcus

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Abstract
Background
Using nanotechnology to develop a new strategy to get beyond the drawbacks of traditional treatments. Among these, metal oxide nanoparticles (MO NPs) produced using herbal resources and environmentally friendly methods have drawn interest due to their possible medical uses. The bioavailability, durability, and capacity to increase cytotoxic effect under ionizing radiation make hafnium oxide nanoparticles (HfO NPs) particularly intriguing. The current work used extracts from Momordica charantia and Vaccinium sect. Cyanococcus to assess the embryonic toxicity of green-synthesized HfO NPs in zebrafish embryos.
Materials and Methods
Extracts from Momordica charantia and Vaccinium sect. Cyanococcus were used in the green synthesis of HfO NPs. In addition to a control group, zebrafish embryos exposed to quantities of 5, 10, 20, 40, and 80 µg/mL were used to characterize and test the nanoparticles for embryonic toxicity. Over the course of 24–96 hours after conception, the hatching rate, viability, and morphological changes of the embryos were evaluated. Tukey's post hoc tests and one-way ANOVA were used for statistical analysis.
Results
At lower doses (5 and 10 µg/mL), both Momordica mediated HfO NPs and Vaccinium mediated HfO NPs had the highest hatching and viability rates, which were comparable to control. Higher concentrations (40–80 µg/mL) were associated with delayed growth, higher mortality, and a substantial decrease in hatching and viability ($p = 0.00$). At favorable concentrations, no significant abnormalities, including axial deformities, pericardial edema, or yolk sac edema, were seen. By using plant extracts as stabilizing agents, HfO NPs were successfully synthesized without the use of hazardous chemicals, as validated by elemental dispersive X-ray spectroscopy.
Conclusion
According to the study on embryonic toxicology, green-synthesised HfO NPs from Momordica charantia and Vaccinium sect. Cyanococcus are biocompatible at lower concentrations and cause little harm to zebrafish embryos during development.At greater concentrations and in a dose-dependent manner, there was higher toxicity highlighting the need of dosage optimization for biomedical applications.

Keywords: Green Synthesised, Hafnium Oxide, Nanoparticles, Embryos, Metal Oxides, Toxicity

Citation: Surabhi Halder, RajeshkumarShanmugam. 2026. Embryonic toxicology evaluation of Hafnium oxide nanoparticles synthesized using Momordica charantia and Vaccinium sect.cyanococcus. FishTaxa 38: 239-248

Introduction
The science and technology of creating new materials at the nanoscale level is known as nanotechnology, and it is expanding quickly. Numerous disciplines, including biology, chemistry, physics, and medicine, have extensive uses for nanotechnology [1]. Furthermore, new therapeutic and diagnostic uses in targeted drug delivery, regenerative medicine, and oncology have been made possible by recent developments in nanotechnology [2,3]. There are two types of nanomaterials: inorganic and organic. The physical, chemical, biological, medicinal, optical, mechanical, and engineering sciences are all very interested in metal and metal oxide nanoparticles. New methods for studying and working with individual atoms and molecules are presented [4]. Metal oxide nanoparticles can be created using a variety of physical and chemical techniques. Nonsputtering, solvothermal, reduction, sol-gel, and electrochemical techniques are a few of the often employed techniques. These techniques are expensive, hazardous, high-pressure, high-energy, and poisonous [5]. Thus, the investigation of green synthesis methods employing microbial agents, plant extracts, and biopolymers has become a viable, economical, and environmentally beneficial substitute [6,7].

An effective and affordable substitute for *in vivo* nanotoxicity screening is the embryonic zebrafish model. This is partly because

zebrafish and humans share a high degree of genetic conservation as well as morphological and physiological similarities, especially during development [8]. Additionally, zebrafish embryos are suitable for moderate-to-high-throughput screening techniques due to their small size, quick growth, and transparency. Along with identifying possible windows of developmental susceptibility to NPs, it is possible to evaluate several routes of nanoparticle exposure, such as ingestion, respiration (gill uptake), and epithelial absorption (primary). Particularly beneficial is the minimal amount of test material needed to examine in vivo toxicity in zebrafish [9]. Furthermore, zebrafish models enhance its applicability as a predictive in vivo system by enabling the simultaneous imaging of morphological changes, organ development, and behavioral abnormalities under nanoparticle exposure [10].

When applying different anti-cancer medicines, the majority of metal oxide nanoparticles are simple to utilize. The main advantage of hafnium oxide nanoparticles' anti-cancer properties is their capacity to generate large amounts of energy inside cancer cells when ionizing radiation activates them, giving them the right amount of bioavailability and persistence [11]. HfO NPs are intriguing candidates for radiotherapy augmentation because, in contrast to other MO NPs, they have been explicitly emphasized for their radiosensitizing actions [1]. The study's novelty is the green synthesis of HfO NPs using two different plant extracts (*Vaccinium sect. Cyanococcus and Momordica charantia)* and the evaluation of their embryonic toxicology in zebrafish as a model [12].

Methods

Vaccinium sect. Cyanococcus powder was brought in the Ayurvedic shop in Poonamallee. 1g of *Vaccinium sect. Cyanococcus* powder was weighed and mixed with 100 mL of distilled water. The mixed solution was placed in the heating mantle for 15 to 20 mins at 50 degree Celsius and the extract was filtered using the muslin cloth and was used to prepare the nanoparticles to check their applications.

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.

One gram of plant extract was weighed in the weighing machine and was thoroughly mixed and it was heated at 60 degrees Celsius for 15 to 20 minutes and filtered. The filtered *Vaccinium Sect. Cyanococcus* and *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 orbital 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.

Zebrafish embryonic toxicology evaluation of HfONPs

a. Fish Maintenance Protocol and Exposure to HfONPs

Under regulated laboratory circumstances, wild-type zebrafish were kept in separate tanks with a light/dark photoperiod of 14:10 hours and an ideal temperature of 28 ± 2 °C. To maintain optimal health and breeding effectiveness, the fish were fed a balanced meal twice a day. Freshly fertilized eggs were taken from each breeding unit after mature breeding partners were permitted to spawn. To guarantee a

sterile environment and eliminate debris, the retrieved eggs were rinsed three times with newly made E3 embryo media (free of methylene blue). Only healthy, fertile eggs were chosen for additional testing.

Embryos were moved into 12-well and 24-well culture plates for the exposure investigation, with 20 embryos in each well containing 2 mL of medium. To guarantee reproducibility, each experimental and control group was made in triplicate. As part of the experimental therapy, embryos were exposed to five distinct doses of Hafnium oxide nanoparticles (HfO NPs) mediated by plant extract. To guarantee even dispersion, the freshly made nanoparticle suspensions in E3 medium were sonicated for 15 minutes. A steady pH range of 7.2–7.3 was maintained by adjusting the medium.

These fluids containing nanoparticles were used to incubate embryos for 24 to 96 hours after fertilization (hpf). Negative controls were untreated embryos cultured in E3 media. The culture plates were kept at 28°C and covered with aluminum foil to prevent photodegradation of the nanoparticles. Every twelve hours, the viability of the embryos was checked, and dead embryos were quickly removed to avoid contamination. In the treatment and control groups, special attention was paid to hatching, developmental progress, and obvious defects.

b. Evaluation of Zebrafish and Its Developmental Stages

A high-definition stereomicroscope was used to continuously observe the zebrafish embryos during the exposure period after

conception. For 24–78 hours after fertilization (hpf), embryos were subjected to five different concentrations of HfO NPs (5, 10, 20, 40, and 80 µg/mL).

At 24-hour intervals, hatching rates and embryo viability were measured, and both the control and treatment groups' developmental progress was meticulously recorded.

The following were the main endpoints assessed:

Hatching/embryo mortality

Rate of hatching success

Frequency and kind of morphological abnormalities (e.g., spinal deformities, delayed growth, pericardial edema, and yolk sac edema).

A COSLAB light microscope with a digital camera was used for photographic documentation in order to take representative pictures of both healthy and impacted embryos.Every 24 hours, the proportion of impacted embryos was measured, and every observation was methodically documented.

Additionally, concentration-dependent developmental toxicity was evaluated by comparing the exposed and control groups. A thorough assessment of the embryonic reactions to nanoparticle exposure was guaranteed by this method.

c. Embryonic toxicology

Prior to 4 hpf (sphere stage), embryos were treated to varying concentrations of HfO NPs, and the developmental results were methodically tracked. The treatment groups were made up of embryos exposed to HfO NPs, while the negative control group consisted of the untreated embryos.

Up to 96 hpf, no discernible differences ($p > 0.05$) in mortality or development were seen at lower concentrations (5, 10, and 20 µg/mL) when compared to controls. On the other hand, mortality increased significantly ($p < 0.01$) in response to exposure to greater quantities (40 and 80 µg/mL). By 96 hpf, mortality was approximately 25% at 80 µg/mL.

Additionally, hatching success depended on focus.Embryos treated with 20 and 40 µg/mL showed a lower hatching rate of about 75% ($p < 0.01$, no significant difference between these two dosages), while control embryos showed 100% hatching. In addition to delayed hatching and reduced growth, hatching rates decreased further and were significantly significant ($p < 0.05$) at 80 µg/mL. Upon morphological evaluation, embryos treated with 5 and 10 µg/mL up to 96 hpf showed no abnormalities. However, anomalies such axial/tail curvature, pericardial edema, fin deformities, tail flexure, spinal cord truncation, and yolk sac edema were noted above 10 µg/mL. At the maximum dose (80 µg/mL) across several developmental stages (24, 48, and 96 hpf), hypoplasia of the head and eyes, a smaller digestive system, and the lack of a swim bladder were also noted.

Toxic synthetic chemicals were avoided by using plant extracts (*Momordica charantia and Vaccinium sect. Cyanococcus*) as stabilizing agents. The cytotoxic, antibacterial, anti-inflammatory, and antioxidant qualities of these extracts also probably helped explain why HfO NPs were biocompatible at lower doses.

In general, tests using zebrafish embryonic toxicology showed that toxicity rises in a dose-dependent fashion. This result emphasizes the necessity of meticulous dose optimization prior to therapeutic use. M. charantia and V. sect. Cyanococcus-mediated HfO NPs may be investigated further as potential treatments for oral squamous cell cancer (OSCC) due to their bioactivity and stabilizing ability. However, prior to therapeutic translation, validation in cancer cell lines and animal models is crucial.

Characterisation of the green synthesised hafnium oxide nanoparticles using Elemental dispersive X-ray spectroscopy.

The elemental composition of green-synthesised hafnium oxide nanoparticles (HfO NPs) was characterized by elemental dispersive X-ray spectroscopy (EDX).To ensure the inclusion of essential components from each plant source, this study was carried out independently for nanoparticles synthesized using extracts from *Momordica charantia and Vaccinium sect. Cyanococcus.*

EDX verified that hafnium was the main element present in *V. sect. Cyanococcus*-mediated HfO NPs, together with oxygen and trace elements. The effective manufacture of oxide-based nanoparticles was confirmed by the expression of the elemental distribution in percentage weight (% wt).

Similarly, EDX analysis revealed that the main constituents of *M. charantia-mediated* HfO NPs were oxygen and hafnium, with minor elements most likely derived from the extract's phytochemicals. The primary and minor elements included in the nanoparticles were clearly shown by the results, which were also presented as percentage weight (% wt).

In addition to avoiding the use of hazardous synthetic chemicals, this analysis demonstrated that both plant extracts worked well as stabilizing and reducing agents during the manufacture of nanoparticles.

Result

1. Estimation of Embryonic Toxicology of *Vaccinium Sect.Cyanococcus* synthesised Hafnium Oxide Nanoparticles

The hatching and survival responses of zebrafish embryos exposed to *Vaccinium sect. Cyanococcus-mediated* hafnium oxide nanoparticles (HfO NPs) at varying concentrations (5, 10, 20, 40, and 80 µg/mL) varied; the embryos were generally viable at all tested concentrations, but the hatching rate showed a concentration-dependent decline when compared to the untreated control group. At the lowest concentration (5 µg/mL), the embryos achieved a 100% hatching rate, which was comparable to the control; this was followed by a progressive decrease in hatching efficiency: 10 µg/mL (80%), 20 µg/mL (75%), 40 µg/mL (60%), and 80 µg/mL (50%). The control group consistently maintained 100% hatching success.

Alongside the hatching data, there was a downward trend in embryo viability as the concentration of nanoparticles increased. 5 µg/mL had the maximum viability (100%), followed by 10 µg/mL and 20 µg/mL, which both maintained 90% viability, and 40 µg/mL, which displayed a similar trend (90%). Viability dramatically decreased to 60% at the highest concentration (80 µg/mL), indicating embryotoxicity caused by nanoparticles.

These findings collectively demonstrate that -synthesised HfO NPs have deleterious impacts on hatching and survival rates at higher doses (40–80 µg/mL), while having negligible harmful effects at lower concentrations (5–20 µg/mL).

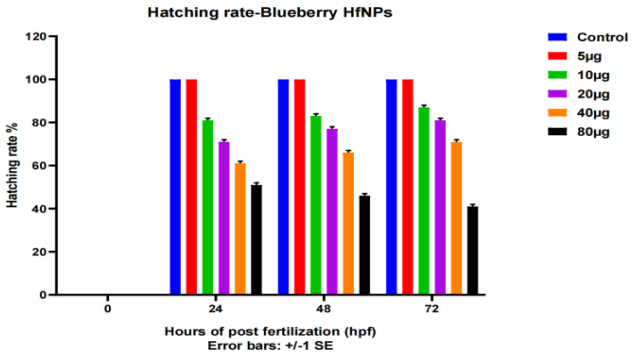

Fig.1:Hatching rate of *Vaccinium* HfNPs

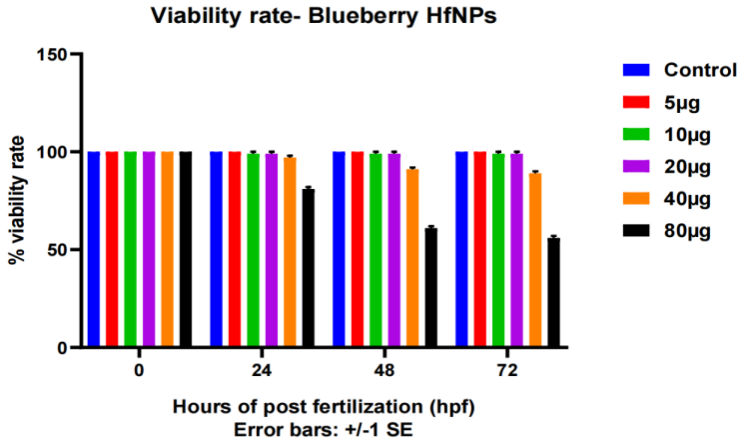

Fig.2:Viability rate of *Vaccinium* HfNPs

Zebrafish embryos treated to varying concentrations of *Vaccinium sect. Cyanococcus*-mediated hafnium oxide nanoparticles (HfO NPs) showed differences in hatching delays. A significantly significant difference in the mortality and viability rates between treatment groups was found by statistical analysis ($p = 0.00$).

The most suitable concentrations were determined by the comparative evaluation to be 5 µg/mL and 10 µg/mL, where the embryos showed the best hatching rates (almost 100%) and maximum viability without any discernible abnormalities.The embryos matured normally and displayed no morphological or structural defects at this concentrations. Crucially, at these ideal concentrations, no axis deformities, tail bending, spinal curvature, yolk sac edema, or pericardial edema—malformations commonly linked to nanoparticle-induced embryotoxicity were observed.

With 100% hatching and viability, embryos kept in the control solution (E3 medium) demonstrated normal development throughout, further confirming the baseline for comparison. According to these results, green-synthesised HfO NPs at lower concentrations (5–10 µg/mL) are safe and biocompatible for zebrafish embryonic development, but larger concentrations carry the danger of embryotoxicity. (Figure 1 & 2)

2. Estimation of Embryonic Toxicology of *Momordica Charantia* synthesised Hafnium Oxide Nanoparticles

When zebrafish embryos were exposed to *Momordica charantia* mediated HfO NPs, their hatching rate decreased in a concentration-dependent manner.While the control group maintained a consistent 100% hatching rate, the maximum hatching success (100%) was observed at 5 µg/mL, followed by 10 µg/mL (95%), 20 µg/mL (78%), 40 µg/mL (62%), and 80 µg/mL (50%).

The viability of the embryos changed with concentration in tandem with the hatching rate. In comparison to the control (100%), the lowest viability was recorded at 80 µg/mL (60%) and the highest viability (100%) was observed at 5 µg/mL, with slightly lower values at 10 µg/mL (90%), 20 µg/mL (90%), and 40 µg/mL (90%). Accordingly, hatching and viability rates peaked at lower concentrations (5–10 µg/mL) and gradually decreased as concentrations increased. (Figure 3)

The treatment groups showed significant differences in hatching delay ($p = 0.00$), validating the dose-dependent embryotoxic effect. Mortality rates significantly rose at greater nanoparticle concentrations, according to a statistical analysis of intergroup data.

These results showed that 5 µg/mL and 10 µg/mL were the most effective concentrations for zebrafish embryonic development, supporting the best hatching success and viability without causing abnormalities. Interestingly, at these concentrations, no morphological or structural problems were seen, including axial deformities, tail bending, spinal curvature, pericardial edema, or yolk sac edema.

Higher doses, on the other hand, caused embryos to hatch later and survive less, suggesting dose-dependent embryotoxic effects.As a trustworthy benchmark, the control group (E3 medium) continuously demonstrated normal embryonic development with 100% hatching and viability rates. (Figure 3 & 4).

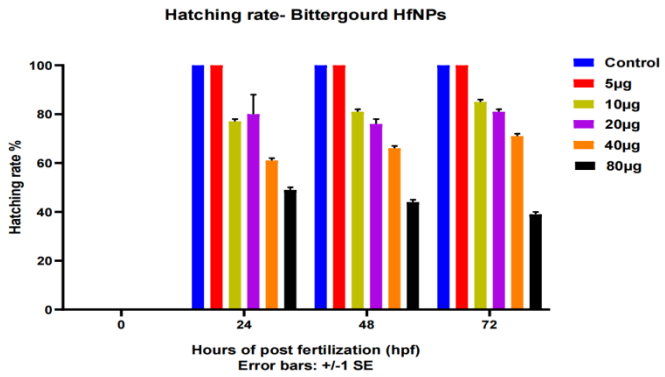

Fig.3: Hatching rate of *Momordica Charantia* HfNPs

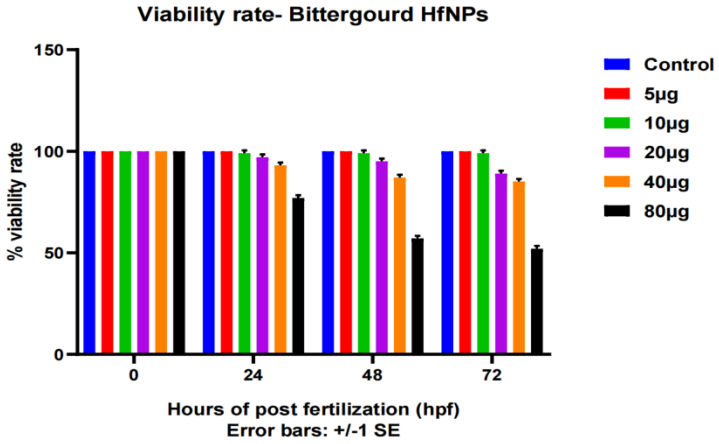

Fig.4: Viability rate of *Momordica Charantia* HfNPs

Fig.5: Developemental stages of Zebra fish Embryos

a. *Vaccinium sect. Cyanococcus* mediated HfO NPs: Effect on Zebrafish Development

The normal development of zebrafish larvae was not affected by exposure to hafnium oxide nanoparticles (HfO NPs) synthesized by *Vaccinium sect. Cyanococcus.* Throughout the trial, the embryos' body length remained normal, and there was no indication of any developmental delays, including prompt absorption of the yolk sac (Figure 5).

The mean body length of zebrafish embryos exposed to the highest tested concentration (80 mg/L) at 72 hours post-fertilization (hpf) was similar to that of the control group, with no statistically significant difference.Likewise, no changes were seen at the lower *Vaccinium*-mediated HfO NP concentrations.

Treatment at both low and high exposure levels (40 and 80 mg/L) had no effect on the area of the yolk sac (mm^2). When compared to controls, the differences were not statistically significant, despite a minor trend of increasing yolk sac area with higher HfO NP concentrations.

In addition, no treatment group showed significant pericardial edema, tail bending, or other morphological abnormalities at any of the amounts under investigation.

Overall, these results indicate that HfO NPs produced by *Vaccinium sect. Cyanococcus* are biocompatible and do not significantly harm zebrafish embryo development, even at the highest doses examined.

b. *Momordica Charantia* mediated HfO NPs: Effect on Zebrafish Development

Zebrafish larvae's normal development was unaffected by exposure to hafnium oxide nanoparticles (HfO NPs) made from *Momordica charantia.* Similar to untreated controls, the embryos had normal body length and prompt yolk sac absorption.

Even high-dose treatment did not hinder larval growth, as evidenced by the fact that the mean body length of zebrafish embryos exposed to the highest measured dosage (80 mg/L) at 72 hours post-fertilization (hpf) remained statistically similar to the control group. Additionally, there were no discernible variations in body length at lower dose levels of *Momordica*-mediated HfO NPs.

However, when compared to controls, the yolk sac area (mm2) increased somewhat at higher concentrations (40 and 80 mg/L), indicating a possible minor physiological reaction at higher dosages. At lesser doses, no discernible variations in yolk sac area were seen, and altogether, the alterations did not point to any pathogenic anomalies.

Developmental abnormalities did not accompany the observed dose-related trend of increased yolk sac area. Crucially, no morphological abnormalities such as spinal curvature, tail bending, or pericardial edema were seen in the treatment group at any concentration (Figure 5).

These findings support the potential of *Momordica charantia* mediated HfO NPs for use in biomedical applications by confirming that they are mostly biocompatible and do not cause detectable developmental toxicity in zebrafish eggs, even at higher doses.

3. Characterisation of Green-Synthesised Hafnium Oxide Nanoparticles (HfO NPs)

a. Elemental Dispersive (EDX) Analysis of *Vaccinium sect. Cyanococcus m*ediated HfO NPs

Utilizing energy-dispersive spectroscopy (EDX), the elemental makeup of vaccine-mediated HfO NPs was ascertained. The resulting EDX spectrum verified that the main constituents of the produced nanoparticles were copper (Cu), oxygen (O), hafnium (Hf), and carbon (C).

According to the quantitative study, the composition was composed of approximately 1.2% copper, 37% oxygen, 21.8% hafnium, and 35% carbon by weight (Wt%). Tiny levels of traces of zinc (Zn), titanium (Ti), and potassium (K) were also found (Figure 6).

With oxygen and hafnium as the main ingredients and stabilizing carbon provided from plants, these results confirm the effective manufacture of hafnium oxide nanoparticles using *Vaccinium sect. Cyanococcus.*

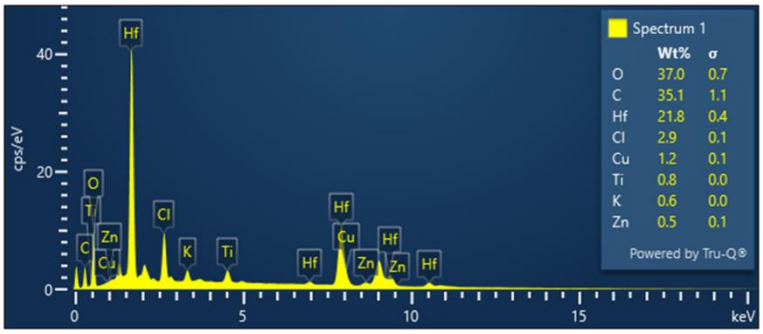

Fig.6 : Elemental Dispersive (EDX) Analysis of *Vaccinium* Mediated HfO NPs

Similarly, the existence of copper (Cu), oxygen (O), hafnium (Hf), and carbon (C) as the primary elements was confirmed by EDX analysis of *Momordica charantia*-mediated HfO NPs.About 1.2% of the composition was copper, 37% was oxygen, 21% was hafnium, and 35% was carbon, according to the compositional breakdown. Furthermore, trace elements such as zinc (Zn), titanium (Ti), and potassium (K) were found (Figure 7).

These distinctive peaks, which have a similar composition to the *Vaccinium*-mediated nanoparticles, further validate the synthesis of HfO NPs utilizing Momordica charantia extract. The presence of carbon indicates that the plant extract's bio-reductive phytochemicals are functioning as organic stabilizers while the nanoparticles are forming.

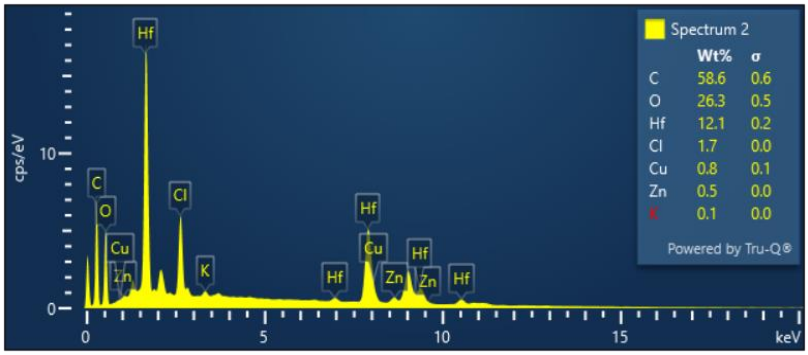

Fig.7 : .Elemental dispersive (EDX) analysis of *Momordica mediated* HfO NPs

Discussion

Nanomaterials are currently widely employed in many other industries, including chemical, electronics, medicines, cosmetics, and many more, as a result of the quick advancement of technology [20]. For instance, titanium dioxide nanoparticles have found extensive application as nanoceramics, coating materials, cosmetic fillers, and catalysts [21][22] [23]. Because of their quick

development, transparency, and genetic resemblance to humans, zebrafish embryos have become a popular vertebrate model as nanotoxicology has advanced. This makes them especially helpful for evaluating developmental and genetic toxicity [24,25]. Zebrafish embryo toxicity assays are now widely used as a standard method to predict the chemical risk of novel nanoparticle formulations toward humans [26].

Zebrafish embryos exposed to green-synthesized HfONPs in the current work showed a definite dose-dependent response. The embryos displayed normal viability, hatching, and development at lower exposure volumes (5–10 µL), whereas poorer hatching and viability rates were linked to higher exposure levels (40–80 µL). The overall patterns observed in Fish Embryo Acute Toxicity (FET) experiments, where negative effects usually manifest in a concentration-dependent way, are in line with this observation [27]. Furthermore, it has been demonstrated that brief exposure to TiO_2 nanoparticles does not significantly damage zebrafish embryos [28], and results also showed that HfONPs did not produce severe developmental abnormalities, even at greater exposure levels. While the yolk sac continued to supply vital nutrients, the chorion's protective function probably limited the penetration of nanoparticles prior to hatching, preventing a major disturbance of embryonic growth [29]. These findings are also consistent with earlier research showing that the chorion barrier and nanoparticle surface chemistry have a significant impact on nanoparticle–embryo interactions [30,31].

Additional information about why these nanoparticles are generally safe at lower dosages can be found in our characterization data. Hafnium and oxygen were identified as the main elements by energy-dispersive X-ray (EDX) analysis, whereas carbon signals were attributed to phytochemical capping agents that were extracted from the plant extracts. According to Farlow [32], trace copper signals were probably artifacts from the copper grids utilized for the measurement.According to Khan [30], these phytochemical coatings have been demonstrated to stabilize nanoparticles and decrease surface reactivity, which lowers nonspecific cellular interactions and lessens toxicity. This is consistent with the lack of sublethal symptoms that are usually thought of as sensitive markers of developmental toxicity, such as axial abnormalities, yolk sac edema, or pericardial edema [33].

When combined, these findings imply that zebrafish embryos tolerate both *Momordica charantia and Vaccinium sect. Cyanococcus* mediated HfONPs well at lower doses, with only minor side effects manifesting at greater exposures. Because hafnium oxide nanoparticles can improve radiation dose deposition at the tumor site, they are currently being investigated as radiosensitizers in cancer therapy, which makes this especially significant in the context of biomedical applications [34]. Green-synthesized HfONPs have the potential to be safe and biocompatible options for translational research because they do not exhibit significant embryotoxicity at physiologically relevant concentrations. Overall, the study offers compelling evidence that plant-mediated HfONPs stabilized by natural phytochemicals have a positive safety profile in zebrafish embryos, supporting additional testing in experimental models relevant to disease [28,30,35].

Limitation

There are still some crucial factors to take into account even if our work shows that green-synthesised hafnium oxide nanoparticles are typically well tolerated at lower doses.To offer a more complete toxicological profile, other endpoints include long-term exposure effects, behavioral changes, detailed evaluation of developmental anomalies, and long-term organ-specific toxicity should be assessed in addition to hatching and mortality rates.Despite being extremely important because of its genetic and physiological resemblance to humans, the zebrafish embryo model nonetheless has certain drawbacks when it comes to extrapolating results to higher vertebrates and clinical settings.For greater predictive accuracy, zebrafish embryotoxicity tests should be viewed as a transitional stage that necessitates supplementary in vitro and in vivo validations.

Conclusion

The Green synthesised hafnium oxide nanoparticles utilizing *Momordica charantia* and *Vaccinium sect. Cyanococcus* demonstrated high biocompatibility, exhibiting normal hatching and viability in zebrafish embryos.The hatching rate was found to be higher in *Vaccinium Sect.Cyanococcus* when compared to *Momordica Charantia* at 24hrs,48hrs and 72hrs.The viability rate was comparable for both *Vaccinium sect.Cyanococcus* and *Momordica Charantia* in different concentrations at 24hrs,48hrs and 72hrs. However, at higher doses, concentration-dependent developmental toxicity was observed.The EDX analysis of vaccinium sect.cyanococcus spectrum verified that the main constituents of the produced nanoparticles were copper (Cu), oxygen (O), hafnium (Hf), and carbon (C).Similarly, the existence of copper (Cu), oxygen (O), hafnium (Hf), and carbon (C) as the primary elements was confirmed by EDX analysis of *Momordica charantia*-mediated HfO NPs.Although further research on long-term safety, molecular insights, and translational validation is crucial, these findings suggest their potential biological applications.

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