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Characterization and Investigation of the Toxicity, Genotoxicity, Antioxidant, and Radical Scavenging Activity of Highly Fluorescent Nitrogen-Doped Graphene Quantum Dots (NGQDs)

Ghavamizadeh, Mehdi[1,2]; Habibi, Azam[1]; Dastghaib, Sanaz[3]; Ahmadi, Mazaher[4]; Mokarram, Pooneh[*+1]

[1]*Department of Biochemistry, School of Medicine, Autophagy Research Center, Shiraz University of Medical Sciences, Shiraz, I.R. IRAN*

[2]*Cellular and Molecular Research Center, Gerash University of Medical Sciences, Gerash, I.R. IRAN*

[3]*Endocrinology and Metabolism Research Center, Shiraz University of Medical Sciences, P.O. Box 71345-1744, Shiraz, I.R. IRAN*

[4]*Faculty of Chemistry and Petroleum Sciences, Bu-Ali Sina University, Hamedan, I.R. IRAN*

ABSTRACT

Nitrogen-Doped Graphene Quantum Dots (NGQDs) have recently been used effectively in drug delivery. Our study aimed to characterize NGQDs and investigate their toxicity and genotoxicity using the Brine Shrimp lethality test (BSLT), Allium cepa L assay, and MTT assay on U251 GBM cells. NGQDs were synthesized using citric acid and Tris(hydroxymethyl) aminomethane through the pyrolysis method. Antioxidant and scavenging activities of NGQDs were also measured. LC50, EC50, and IC50 of NGQDs were determined; the Allium cepa L assay was also used to explore the potential mutagenic and carcinogenic effects of NGQDs. NGQDs had an average size of 8.1±1 nm with a zeta(ζ)-potential of −24.4 mV. ATR FT-IR findings demonstrate that NGQDs have functional groups, and TEM analysis revealed heterogeneous morphologies. AFM measurements indicate that the size of the distribution of NGQDs is relatively uniform except for some agglomeration of NGQDs. UV-vis and FL spectra were 335 and 410 nm, respectively. ABTS+ and DPPH radical scavenging activities were 4.13±0.4 and 61.32±0.22%, respectively. Total phenol content and antioxidant power were 23±1.15 µg GAE/g and 577.98±0.28 µmol Fe^{2+}/g, respectively. LC50, EC50, and IC50 values were 3388.4±224.3, 5825.4±703.3, and 3057±156.9 µg/mL, respectively. In conclusion, NGQDs exhibited high photoluminescence and biocompatibility with no significant cytotoxic or genotoxic effects, making them highly safe for drug delivery. Moreover, it was concluded that the BSLT and Allium cepa L assay could be good alternatives to the MTT assay though further research is needed to confirm this.

ARTICLE INFO

Keywords

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**To whom correspondence should be addressed.*

+ E-mail: mokaramp@sums.ac.ir

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Abbreviations

NGQD, nitrogen-doped graphene quantum dot; NPs, nanoparticles; BSLT, brine shrimp lethality test; DLS, dynamic light scattering; FT-IR, Fourier-transform infrared spectroscopy; TEM, transmission electron microscopy; UV–Vis, ultraviolet-visible absorption spectroscopy. TPC, total phenolic content; FL, Fluorescence; PL, photoluminescence; FRAP, ferric reducing antioxidant power; F-C, Folin-Ciocalteu; RSA, radical scavenging activity; ABTS+, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; LC50, half maximal lethal dose; EC50, half maximal effective concentration; GAE, Gallic acid equivalent; DMSO, Dimethyl-sulfoxide; EDTA, Ethylene diamine tetra-acetic acid. CA, citric acid anhydrous; Tris HMA, tris (hydroxymethyl) aminomethane; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IC50, Half-maximal inhibitory concentration; CI, confidence interval. GBM, Glioblastoma.

Statistical analysis

All experiments were performed in triplicate. Data were expressed as mean ± S.D. Statistical analyses were performed using IBM SPSS Statistics 20, GraphPad Prism version 8.0, and Minitab 16 Statistical Software. Comparisons between groups were performed using one-way ANOVA with Tukey's post hoc test. Kruskal-Wallis's test was also used to compare the nonparametric data. $P<0.05$ was considered statistically significant.

Ethical consideration

This paper has been extracted from the Ph.D. thesis of Mehdi Ghavamizadeh, supported by Shiraz University of Medical Sciences (29102), with an ethical code of IR.SUMS.REC.1403.025.

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REFERENCES

[1] Ghosh D., Sarkar K., Devi P., Kim K.-H., Kumar P., Current and Future Perspectives of Carbon and Graphene Quantum Dots: From Synthesis to Strategy for Building Optoelectronic and Energy Devices, *Renewable and Sustainable Energy Reviews*, **135:** 110391 (2021).

[2] Madhi A., Shirkavand Hadavand B., Madhi A.H., Bio-Friendly Fluorescent Polyvinyl Alcohol/Gelatin/Chitosan Hydrogel Membranes Strengthened by G-C3N4/Cqds Nanocomposite: Preparation, Investigation of UV-Absorption, Mechanical and Rheological Properties, *Fullerenes, Nanotubes and Carbon Nanostructures*, **32(6):** 611-620 (2024).

[3] Madhi A., Shirkavand Hadavand B., Madhi M.H., Smart Fluorescent PVA/CS/AV/G-C3n4qds Hydrogel Nanocomposites: Synthesis and Study of UV Absorbance, Rheological and Self-Healing Properties, *Fullerenes, Nanotubes and Carbon Nanostructures*, **32(9):** 806-816 (2024).

[4] Pierrat P., Gaumet J.-J., Graphene Quantum Dots: Emerging Organic Materials with Remarkable and Tunable Luminescence Features, *Tetrahedron Letters*, **61(49):** 152554 (2020).

[5] Li S., Li L., Tu H., Zhang H., Silvester D.S., Banks C.E., Zou G., Hou H., Ji X., The Development of Carbon Dots: From the Perspective of Materials Chemistry, *Materials Today*, **51:** 188-207 (2021).

[6] Perini G., Palmieri V., Ciasca G., D'Ascenzo M., Gervasoni J., Primiano A., Rinaldi M., Fioretti D., Prampolini Ch., Tiberio F., Lattanzi W., Parolini O., De Spirito M., Papi M.., Graphene Quantum Dots' Surface Chemistry Modulates the Sensitivity of Glioblastoma Cells to Chemotherapeutics, *International Journal of Molecular Sciences*, **21(17):** 6301 (2020).

[7] Sengupta S., Pal S., Pal A., Maity S., Sarkar K., Das M., A Review on Synthesis, Toxicity Profile and Biomedical Applications of Graphene Quantum Dots (Gqds), *Inorganica. Chimica. Acta.*, **557:** 121677 (2023).

[8] Kersting D., Fasbender S., Pilch R., Kurth J., Franken A., Ludescher M., Naskou J., Hallenberger A., von Gall Ch., Mohr C.J., Lukowski R., Raba K., Jaschinski S., Esposito I., Fischer J.C., Fehm T., Niederacher D., Neubauer H., Heinzel Th., From in Vitro to Ex Vivo: Subcellular Localization and Uptake of Graphene Quantum Dots Into Solid Tumors, *Nanotechnology*, **30(39):** 395101(2019).

[9] Iannazzo D., Pistone A., Salamò M., Galvagno S., Romeo R., Giofré S.V., Branca C., Visalli G., Di Pietro A.N., Graphene Quantum Dots for Cancer Targeted Drug Delivery, *International Journal of Pharmaceutics*, **518(1-2):** 185-192 (2017).

[10] Lee B.-C., Lee J.Y., Kim J., Yoo J.M., Kang I., Kim J.-J., Shin N., Kim D.J., Choi S.W., Kim D., Hong B.H., Kang K.-S., Graphene Quantum Dots as Anti-Inflammatory Therapy for Colitis, *Science Advances*, **6(18):** Eaaz2630 (2020).