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Anti-Angiogenesis effect of Graphene-loaded Green Synthesized Zinc Oxide Nanoparticles on Chick Chorioalantoic Membrane

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Abstract

Graphene and Zinc Oxide nanoparticles (ZnO-NPs) have unique properties in the diagnosis and treatment of diseases. By loading ZnO-NPs on graphene, their biological compatibility can be greatly improved. In the present study, we investigated the anti-angiogenesis properties of ZnO-NPs synthesized using Crocus Sativus petal extract loaded on graphene (CS/ZnO-NPs/G) on chick Chorioallantoic Membrane (CAM). The results showed that CS/ZnO-NPs/G reduced significantly the number and length of blood vessels, crown roost length and weight in chick embryo chorioalantoic membrance compared to control. The findings suggest that CS/ZnO-NPs have inhibitory effect on angiogenesis and can be promising to treat many diseases, such as cancer and chemotherapy.

Keywords: Angiogenesis, Zinc Oxide, Graphene, Chorioallantoic Membrane.

Introduction

Nanotechnology has been considered as an applied technology in recent decades. This technology creates the ability to work at the level of metal oxides, carbon atoms, and the creation of structures that have a completely new molecular order. Among the metal oxides, Zinc Oxide is considered to be one of the best exploited at nanodimensions (Sabir et al., 2014) and has several unique properties, such as high electron mobility, strong room temperature luminescence (Azarang et al., 2014), biocompatible and environmentally friendly (Rasmussen et al., 2011), (Zaman, 2012). Zinc Oxide is also important as a semiconductor with wide band gap of 3.3 ev at room temperature (An et al., 2014), which is important for scientific and industrial applications. During the work, we used ZnO-NPs synthesized using Crocus Sativus petal extract; the green-synthesized nanoparticles have weak toxicity or non-toxicity. It can therefore be used to treat cancer and selective killer cancer cells (Hu et al., 2013).

Graphene is the newest member of the family of carbon multidimensional materials(Sederberg, 2009) having a honeycomb structure (Labhane et al., 2016) , (Lim, 2017) and unique properties including low toxicity (Jiao et al., 2015), (Han & Kim, 2013), high chemical stability, surface to high volume ratio and optical and electrical features are exceptional (Rethinasabapathy et a l., 2017). Unique applications in the biological field, diagnosis and treatment of diseases provide.

Due to the high properties of graphene and ZnO-NPs, by loading ZnO-NPs synthesized from the Crocus Sativus petal extract on graphene, their anti-cancer properties can be significantly increased (Haldorai et al., 2014). Recently, studies have been conducted on the anti-angiogenesis of nanoparticles to inhibit angiogenesis and inhibit metastases. Angiogenesis is an important biological mechanism that

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involves the formation of new blood vessels from pre-existing ones (Barui et al., 2012). Angiogenesis in mammals also occurs during the reproductive cycle, pregnancy (Wierzbicki et al., 2013), embryo development, tissue regeneration, wound repair, and the return of blood flow to the damaged tissue (Shah et al., 2015), which results in the formation of new blood vessels provides oxygen and nutrients to the growing cells that are capable of rebuilding the tissue (Ahtzaz et al., 2017). The angiogenesis process depends on the balance between pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF-2) and anti-angiogenic agents such as angiostatin, angiopoietin 2 and andstatin. In the event of imbalance between them, the conditions for the occurrence of certain diseases are created. Decreased angiogenesis in cardiovascular diseases and ischemia diseases (Barui et al., 2012), neurodegenration, hypertension and respiratory distress are seen. Excessive angiogenesis leads to cancer, psoriasis, arthritis, diabetic blindness, asthma, and atherosclerosis. In this condition, new blood vessels feed on the patient's tissues and destroy normal tissues (Grodzik et al., 2011). The creation of new vessels for the formation, development and metastasis of tumors is essential. Angiogenesis in the tumor suppresses tumor growth and prevents metastasis and is a key target in cancer treatment. Various studies have been done to control angiogenesis. Some studies have shown that metal oxide and carbon nanomaterials inhibit angiogenic signaling pathways with less toxicity and therefore can be potentially used to treat cancer to reduce angiogenesis (Ahtzaz et al., 2017), (Wierzbicki et al., 2013). So far, studies have not been conducted on the anti-angiogenesis activity of CS/ZnO-NPs/G on chick chorioallantoic membrane. It is necessary to identify new therapeutic molecules that may significantly increase angiogenic inhibition. Therefore, the purpose of this study was to evaluate anti-angiogenesis activity of CS/ZnO-NPs/G in vivo.

Materials and Methods

Chorioalantoic membrane assay

In this research, thirty fertilized Lean races chicken eggs, purchased from Murgak Mashhad Company (Iran), were carefully cleaned with 70% alcohol and randomly divided into 3 experimental groups. Group 1was control and kept under normal conditions (without any treatment) and two other groups were experimented (treated with concentrations of 125, 250 and 500 µg / ml CS/ZnO-NPs/G). Then, the eggs were inserted in an automatic rotation Incubator at 38 ° C and 70-65% humidity. After 48 h of the incubation, egg shells were opened under laminar flow hood. On the seventh day of incubation, the injection of proper concentration of CS/ZnO-NPs/G was performed using a shell insulin syringe on the chorioallantoic membrane under sterile conditions. The injection volume was 125, 250 and 500 microgram per ml for each sample. After injection, the holes were blocked by sterile paraffin (Paraffin Fara, Iran) and the eggs were then transferred to the incubator. At days 10 of incubation, part of the injection shell was removed and CAM were carefully separated and examined with microscope. The stereomicroscope instrument was used to taking photo for analyzing the length and number of blood vessels in chorioallantoic membrane. Measurements were repeated 3 times to prevent the error. Quantitative data from vascular counting and measurement were analyzed by SPSS software using t-test and one way ANOVA at the significance level $P < 0.05$.

Results

Antiangiogenic effect of CS/ZnO-NPs/G

The results of aplication of CS/ZnO-NPs/G in the chick embryo chorioallantoic membrane on the 10th day of incubation show its inhibitory effects on angiogenesis, the results of which are shown in Fig. 1.

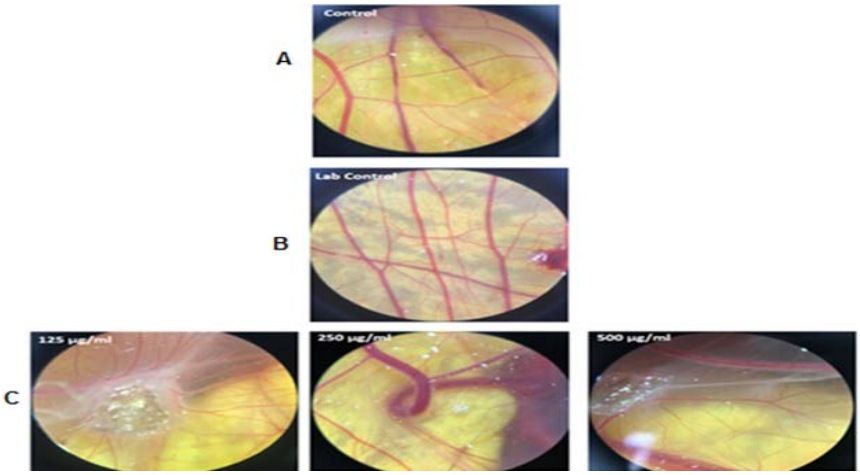

Fig. 1: Image of CAM on the 10th day of incubation. A) Control group, b) Laboratory control group, c) Treatment group

Measure the length and number of blood vessels

There was no significant difference between the mean number (11.525 cm) and length (30.74) of vascular splits in control samples with mean number (10.875) and length (31.825) vascular splits in laboratory samples (P> 0.05). In the next step, the samples of the treatment groups were compared with the control samples. Comparison of mean number and length of vascular splits in the control group with number and length of branches in the treated group with minimum concentration (125 µg / ml) showed a significant difference in the number of vessels (P <0.05), and also there was a significant difference during the vessel (P <0.01). The number and length of branches in the treated sample with a concentration of 250 µg / ml were significantly reduced compared to the control sample (P <0.01). Comparison of mean number and length of branches in the treated sample with the highest concentration (500 µg / ml) had a significant decrease compared to the control sample (P <0.001). It seems that CS/ZnO-NPs/G have anti-angiogenesis effects.

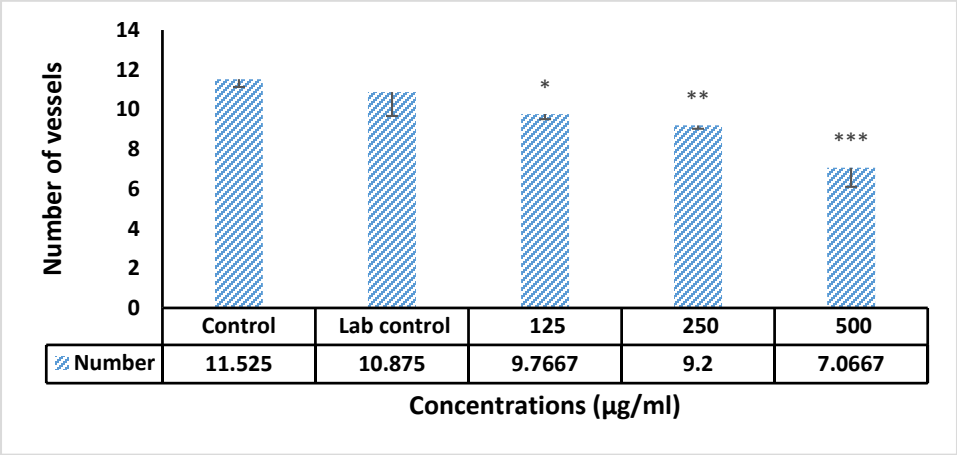

Fig. 2: Mean number of blood vessel branching in samples treated with CS/ZnO-NPs/G compared to control group (***P<0.001, **P<0.01, *P<0.05)

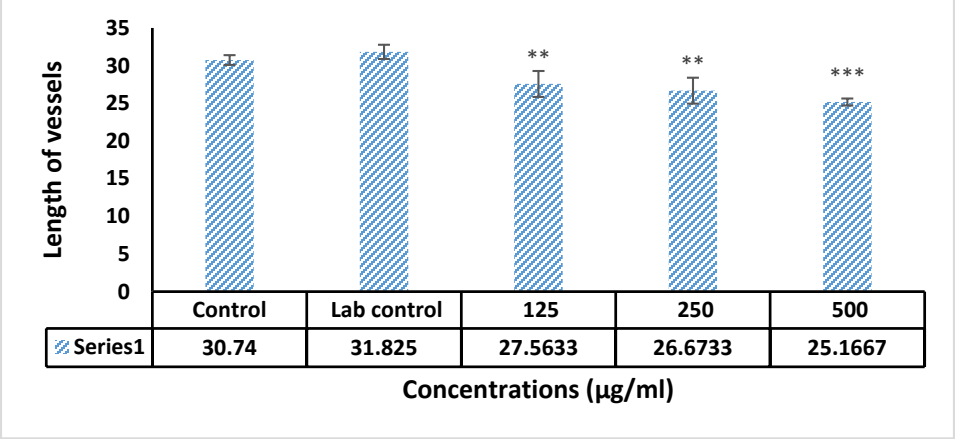

Fig. 3: Mean length of blood vessel branching in samples treated with CS/ZnO-NPs/G compared to control group

Measure the crown roost length and weight

There was no significant difference in the mean weight of chick embryos in the control and control groups (P> 0.05). However, in subsequent studies, the samples of the treatment groups were compared with the control samples. Comparison of mean crown roost length and weight in the control group and the treated group with minimum concentration (125 µg / ml) showed a significant difference during the samples (p <0.01) and also, there was a significant difference in weight of samples (P <0.05). Crown roost length and weight in the samples treated with 250 µg/ml concentration were significantly different (P <0.001) compared to the control sample (P <0.001). Also, there was a significant difference in the weight of the samples (P <0.01). Comparison of mean crown roost length and weight in the treated sample with the highest concentration (500 µg / ml) had a significant decrease compared to the control sample (P <0.001).

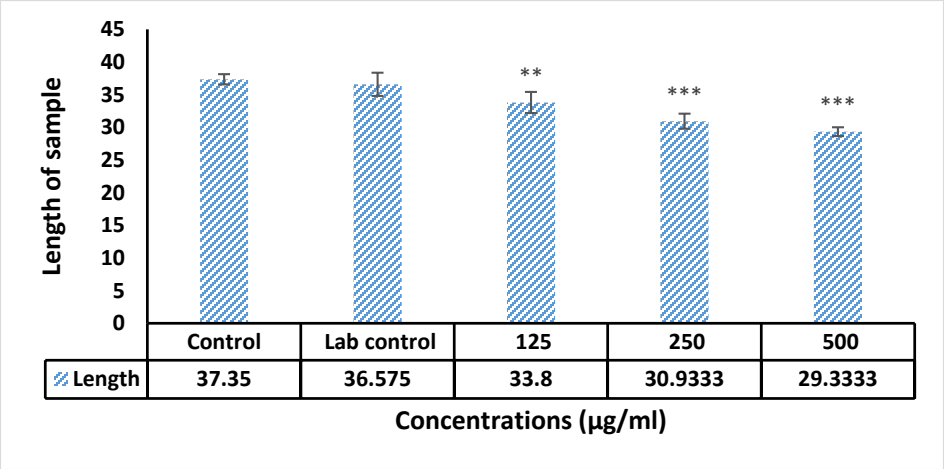

Fig. 4: mean crown roost length in samples treated with CS/ZnO-NPs/G compared with control samples

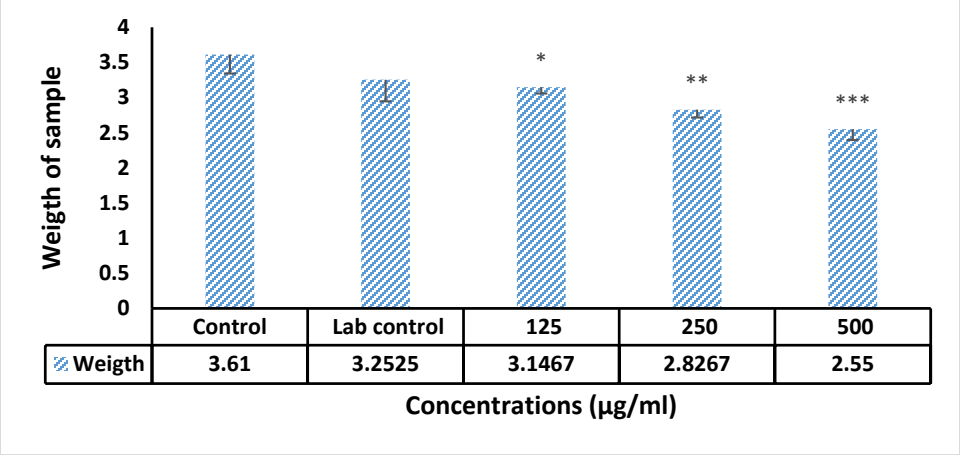

Fig. 5: mean crown roost weight in samples treated with CS/ZnO-NPs/G compared with control samples

Discussion

Since angiogenesis is an important step in the development and metastasis of cancer, many efforts have been made to prevent angiogenesis. Treatments that directly target angiogenesis have a lower risk of resistance to drugs, and in this regard, the problem of resistance to tumors is reduced to treatments. Nanotechnology is also involved in the synthesis of various nanoparticles and is used to control angiogenesis, but the results are varied (Sanaeimehr et al., 2018) . In this study, the angiogenesis effects of CS/ZnO-NPs/G were investigated. The results of this study showed that the use of CS/ZnO-NPs/G significantly decreased the number and length of blood vessels, crown roost length and weight in the chick embryo chorioallantoic membrane compared with control samples. The results indicate that CS/ZnO-NPs/G reduce expression of the angiogenesis stimulator factor (vascular endothelial growth factor) in cancer cells. Previous studies have shown that ZnO-NPs loaded on chitosan and cellulose based on hydrogels have significant blood vessel growth, confirming the angiogenic nature of these substances, and the results of the blood vessel network proved that ZnO-NPs increases angiogenesis and ZnO-NPs generates active oxygen species (H2O2), which may lead to angiogenesis progression in samples treated with ZnO-NPs loaded onto the hydrogel (Ahtzaz et al., 2017). In reseach, in vivo, polyvinyl alcohol / carboxymethyl cellulose scaffolds loaded onto reduced graphene oxide nanoparticles significantly increased angiogenesis(Chakraborty et al., 2018). In another study, copper nanoparticles from copper nanoparticle decorated reduced graphene oxide (RGO Cu) hybrid can stimulate endothelial cells and thus increase angiogenic potential, in fact, the addition of copper ions leads to fusion of small tubullar structures into long tubular networks. The formation of long tubes with larger branches in vitro by endothelial cells in the presence of copper nanoparticle decorated reduced graphene oxide (RGO Cu) hybrid exhibits higher angiogenesis potential (Jaidev et al., 2017). According to previous studies, graphene does not have angiogenesis properties and has no effect on the growth of embryos (body weight, organs), as well as the biochemical indices of chick embryos(Kurantowicz et al., 2017) , (Wierzbicki et al., 2013). Various results have been reported on the angiogenesis and anti-angiogenesis of ZnO-NPs. Some studies have shown that ZnO-NPs stimulate vascular endothelial growth factor and activate angiogenesis(Ahtzaz et al., 2017),(Barui et al., 2012). In a study to investigate the anti-angiogenesis effects of ZnO-NPs

synthesized from marine seaweed extract on chick embryo chorioallantoic membrane, ZnO-NPs stopped forming blood vessels and had inhibitory effects on angiogenesis (Sanaeimehr et al., 2018). In the present study, given that the CS/ZnO-NPs/G showed anti-angiogenesis effects, it is assumed that the results are based on anti-angiogenesis effects of ZnO-NPs, and it is expected that some of the inhibitory effects of ZnO-NPs due to graphene coating. Therefore, the anti-angiogenesis properties of CS/ZnO-NPs/G can be promising to treat many diseases, such as cancer and chemotherapy.

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Compliance with Ethical Standards

Conflict of Intrest: The authors declare that they have no conflict of interest.

Abbreviations

Zinc Oxide nanoparticles, ZnO-NPs; CS/ZnO-NPs/G, ZnO-NPs synthesized using Crocus Sativus petal extract loaded on graphene; CAM, Chick Chorioallantoic Membrane

References

Ahtzaz, S., Nasir, M., Shahzadi, L., Iqbal, F., Chaudhry, A. A., Yar, et al. (2017). A study on the effect of zinc oxide and zinc peroxide nanoparticles to enhance angiogenesis-pro-angiogenic grafts for tissue regeneration applications. Materials and Design, 132, 409–418.

An, S., Joshi, B. N., Lee, M. W., Kim, N. Y., & Yoon, S. S. (2014). Applied Surface Science Electrospun graphene-ZnO nanofiber mats for photocatalysis applications. Applied Surface Science, 294, 24–28.

Azarang, M., Shuhaimi, A., Yousefi, R., Moradi Golsheikh, A., & Sookhakian, M. (2014). Synthesis and characterization of ZnO NPs/reduced graphene oxide nanocomposite prepared in gelatin medium as highly efficient photo-degradation of MB. Ceramics International, 40, 10217–10221.

Barui, A. K., Veeriah, V., Mukherjee, S., Manna, J., Patel, A. K., Patra, et al. (2012). Zinc oxide nanoflowers make new blood vessels. Nanoscale, 4(24), 7861–7869.

Chakraborty, S., Ponrasu, T., Chandel, S., Dixit, M., & Muthuvijayan, V. (2018). Reduced graphene oxide-loaded nanocomposite scaffolds for enhancing angiogenesis in tissue engineering applications. Royal Society Open Science, 5(5), 172017.

Grodzik, M., Sawosz, E., Wierzbicki, M., Orlowski, P., Hotowy, A., Niemiec, et al. (2011). Nanoparticles of carbon allotropes inhibit glioblastoma multiforme angiogenesis in ovo. International Journal of Nanomedicine, 6, 3041–3048.

Haldorai, Y., Voit, W., & Shim, J. J. (2014). Nano ZnO@reduced graphene oxide composite for high performance supercapacitor: Green synthesis in supercritical fluid. Electrochimica Acta, 120, 65–72.

Han, J. W., & Kim, J. (2013). Green synthesis of graphene and its cytotoxic effects in human breast cancer cells. International Journal of Nanomedicine,8, 1015–1027.

Hu, Z., Li, J., Li, C., Zhao, S., Li, N., Wang, et al. (2013). Folic acid-conjugated graphene–ZnO nanohybrid for targeting photodynamic therapy under visible light irradiation. Journal of Materials Chemistry B, 1(38), 5003.

Jaidev, L. R., Kumar, S., & Chatterjee, K. (2017). Multi-biofunctional polymer graphene composite for bone tissue regeneration that elutes copper ions to impart angiogenic, osteogenic and bactericidal properties. Colloids and Surfaces B: Biointerfaces, 159, 293–302.

Jiao, G., He, X., Li, X., Qiu, J., Xu, H., Zhang, et al. (2015). Limitations of MTT and CCK-8 assay for evaluation of graphene cytotoxicity. RSC Adv., 5(66), 53240–53244.

Kurantowicz, N., Sawosz, E., Halik, G., Strojny, B., Hotowy, A., Grodzik,et al. (2017). Toxicity studies of six types of carbon nanoparticles in a chicken-embryo model. International Journal of Nanomedicine, 12, 2887–2898.

Labhane, P. K., Patle, L. B., Huse, V. R., Sonawane, G. H., & Sonawane, S. H. (2016). Synthesis of reduced graphene oxide sheets decorated by zinc oxide nanoparticles : Crystallographic , Optical, morphological and photocatalytic study. Chemical Physics Letters, 661, 13–19.

Lim, H. (2017). Highly Efficient Preparation of ZnO Nanorods Decorated Reduced Graphene Oxide Nanocomposites. Materials Letters, 80, 9–12.

Rasmussen, J. W., Martinez, E., Louka, P., & Wingett, D. G. (2011). Zinc Oxide Nanoparticles for Selective Destruction of Tumor Cells and Potential for Drug Delivery Applications. NIH Public Accsess, 7(9), 1063–1077.

Rethinasabapathy, M., Kang, S., Jang, S., & Huh, Y. S. (2017). Three-dimensional porous graphene materials for environmental

applications, Carbon Letters, 22, 1–13.

Sabir, S., Arshad, M., & Chaudhari, S. K. (2014). Zinc Oxide Nanoparticles for Revolutionizing Agriculture : Synthesis and Applications, Hindawi Publishing Corporation. https://doi.org/10.1155/2014/925494

Sanaeimehr, Z., Javadi, I., & Namvar, F. (2018). Antiangiogenic and antiapoptotic effects of green-synthesized zinc oxide nanoparticles using Sargassum muticum algae extraction. Cancer Nanotechnology, 9(3).

Sederberg, D. (2009). Allotropes of Carbon : It ' s all in the way you ' re put together. NOLT,1–25.

Shah, B. U. H., Gonjari, G. R., & Patil, A. E. (2015). Influence Evaluation of Ocimum Sanctum Leaf Extract on Angiogenesis by using Chick Chorioallantoic Membrane (CAM) Assay. Global Journal of Science Frontier Research: C Biological Science, 15(4), 918–925.

Wierzbicki, M., Sawosz, E., Grodzik, M., Prasek, M., Jaworski, S., & Chwalibog, A. (2013). Comparison of anti-angiogenic properties of pristine carbon nanoparticles. Nanoscale Research Letters, 8(1), 1–8.

Zaman, S. (2012). Synthesis of ZnO , CuO and their Composite Nanostructures for Optoelectronics , Sensing and Catalytic Applications. Linköping Studies in Science and Technology,1467.