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CHEMOPREVENTIVE EFFECTS OF PHENETHYL ISOTHIOCYANATE IN A GRAPHENE OXIDE BASED NANOCOMPOSITE AGAINST HUMAN LUNG, BREAST AND COLON CANCER CELL LINES

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ABSTRACT

Nanocomposites are on the trials in research to be used as potential carriers of anticancer compounds. In this study, graphene oxide (GO) was used as the nanocomposite carrier of tested anticancer compound phenethyl isothiocyanate (PEITC). The objective of this research is to evaluate the chemopreventive effects of designed nanocomposite GO-PEITC on human lung, breast and colon cancer cell lines. The formulated nanocomposite drug (GO-PEITC) along with the pure compound PEITC, and GO were tested on the cell viability of A549 (Lung cancer cells), MCF-7 (Breast cancer cells), and HT-29 (Colon cancer cells) for 72 hours in increasing concentrations (0 – 10 µg/ml). The results showed chemopreventive effects of the formulated nanocomposite (GO-PEITC) on all three human cancer cell lines in an increasing dose pattern. It was clear from the results that the nanocomposite drug has better anti-cancer effects on the cancer cells as compared to the pure drug (PEITC). Therefore, this

research can be used as a baseline to develop PEITC as a GO-based nanocomposite drug with strong chemopreventive properties for cancer treatment upon conducting thorough pre-clinical and clinical trials.

KEYWORDS: Phenethyl isothiocyanate, graphene oxide, chemopreventive, nanocomposite, drug nanocarrier.

INTRODUCTION

Cancer is considered the worst disease in humans that remains a challenge for treatment and therapy. Research efforts are increasing tremendously to find a potential solution for cancer (Subramani et al., 2017). Various drugs and formulations have been tested and most of the drugs do not pass the bioavailability test. This is due to several reasons like delicate physical structure of drugs, target tissues in human body, and the compatibility of drug compounds with the body's transport and absorption systems (Harris et al., 2011; Dorniani et al., 2016). To overcome this problem, research efforts are emphasized on producing nanocarriers for the drugs to protect and deliver them to target tissues without losing their physical and chemical properties (Meyer-Gerspach et al., 2014). The nanocarriers also need to have physiochemical compatibility with the drug to avoid any interaction or chemical reaction that could decrease the drug's therapeutic potential (Rosas et al., 2017). Different methods and types of nanocarriers are being tested to optimize the best results in anti-cancer drug delivery. Among the nanocarrier agents employed in research for treatment and therapeutic potentials of anti-cancer drug compounds are polymeric nanoparticles, liposomes, dendrimers, carbon nanotubes, and so on (Ali et al., 2018). Drugs are commonly attached to their transporting nanocarriers through embedding, hydrogen-bonding, physical loading, and few other methods to increase the half-life of the drug and sustain their release at target tissues upon a longer period of time (Dong et al., 2016). In that case, graphene and its derivative graphene oxide (GO) has gained much interest recently as nanocarriers for drugs due to their high thermal and electric conductivity, enormously large surface area, easy functionalization through π-π interactions, and so on (Li et al., 2018). The biomedical applications of graphene and its derivatives can be improved with addition of various polymers (Ananya and Vimala, 2018).

Plants are well-known for their antioxidant capacity. Cruciferous vegetables contain major bioactive compounds known as isothiocyanates. Isothiocyanates have been studied for their anti-cancer properties on various types of cancer especially lung, breast, colorectal, kidney, prostrate, and pancreatic cancers (Boreddy et al., 2011; Gupta et al., 2014). Although all the

studies have shown positive correlation between the amount of isothiocyanates intake and their anticancer effects, there are several studies that showed inverse effects depending on the duration of intake and age group of patients. Among many isothiocyanates, phenethyl isothiocyanates (PEITC) are the most proven to have anticancer properties with strong pharmacokinetic profile and has been taken for clinical trials. PEITC can be naturally found as gluconasturtiin in cruciferous vegetables like broccoli, water cress, turnips, and radish. Chemopreventive effects of PEITC has described through in-vivo animal model having multiple-chemically induced cancer models (Singh et al., 2012; Aras et al., 2013). PEITC is recognized to exert chemopreventive mechanism through alteration of phase I and phase II metabolizing enzymes thus preventing the bioactivation of chemicals responsible for inducing cancer. Other than that, PEITC has also exerted chemopreventive and chemotherapeutic effects through triggering of apoptosis in cancer cells, modification of proteins specific for cancer cell proliferation, targeting cancer specific biomarkers, regulation of reactive oxygen species (ROS) as the etiology of cancer progression, and other effects in both in-vitro and in-vivo pre-clinical trials (Gupta and Srivastava, 2010; Wu et al., 2010; Mi et al., 2011). Drug resistance is a major obstacle in clinical treatment of cancer leading to poor outcome. There are several causes for drug resistance which includes increased efflux of drugs, reduced drug uptake, reversal of DNA damage through repair mechanisms, inactivation of drugs by metabolizing enzymes, and introduction of anti-apoptotic mechanism by cancer cells (Saw et al., 2011; Gupta et al., 2013). PEITC has been proven to modify a few among these drug resistance factors hence a complete administration of PEITC at cancer sites is required through enhanced bioavailability of the drug. This can be achieved with the recent nanocarriers technology by loading anticancer drug into nanocarriers and delivering them at target sites by enhancing the drug release sustainability (Fuentes et al., 2017). Therefore in this research, we aim to formulate a nanocomposite GO-PEITC drug to assess its chemopreventive effects on human lung cancer (A549), breast cancer (MCF-7), and colon cancer (HT-29) cell lines.

MATERIALS AND METHODS

Chemicals

Phosphoric acid (H_3PO_4), Sulfuric acid (H_2SO_4 98%), Graphite flakes (109 meshes), Phenethyl isothiocyanate (PEITC), Hydrogen peroxide, Potassium permanganate ($KMnO_4$), and Phosphate buffered saline (PBS) were obtained from Sigma Aldrich (St Louis, MO, USA). 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-tetrazolium bromide (MTT), Hydrochloric

acid (HCl 37%), Ethyl Alcohol (99.7% v/v), Diethyl ether, Sodium hydroxide, Dulbecco's modified eagle medium (DMEM), Heat inactivated fetal bovine serum (FBS), Antibiotics penicillin- streptomycin were purchased from Friedemann Schmidt (WA, USA).

Cell lines and culture conditions

Human lung cancer (A549), breast cancer (MCF-7), and colon cancer (HT-29) cell lines were cultured under normal conditions in Dulbecco's modified Eagle's medium (DMEM) with 95% humidified air and 5% carbon dioxide at 37°C, supplemented with 1% mixture of antibiotics (penicillin- streptomycin) and 10% heat inactivated fetal bovine serum. The cells were then sub-cultured in 75 cm^2 culture flasks and used for treatment or seeding upon reaching 80-90% confluence.

Graphene oxide synthesis

The synthesis of Graphene oxide (GO) was done following the modified method of Marcano et al. (2010). Briefly, 360 mL of concentrated sulfuric acid was added to 40 mL of concentrated phosphoric acid and the solution was mixed with 18 g of potassium permanganate and 3 g of graphite powder. The mixture was stirred for 12 hours at 50°C using hot plate stirrer. The resulting liquid was poured onto ice cubes (400 g) containing hydrogen peroxide (3 mL). Then the solution was rinsed with deionized water (200 mL), hydrochloric acid (200 mL), and ethyl alcohol (200 mL). Finally, diethyl ether was used to coagulate the solution and dried at 40°C.

Loading of phenethyl isothiocyanate (PEITC) into GO

Briefly, PEITC (2 mL) was thoroughly dissolved in ethanol (100 mL) and the solution was added with GO (0.5 g). The mixture was stirred continuously for 24 hours, then centrifuged, washed, and dried at 40°C. The nanocomposite formed (GO-PEITC) was subjected to physiochemical characterization (results not shown) using X-ray diffraction (XRD), high resolution transmission electron microscope (HR-TEM), and Infrared Spectroscopic analysis.

Determination of anticancer assay (MTT assay)

Chemopreventive effects of the formulated nanocomposite GO-PEITC along with the pure form of GO and PEITC were evaluated on human lung cancer (A549), breast cancer (MCF-7), and colon cancer (HT-29) cell lines using mitochondrial dependent reduction of MTT to formazan colorimetric analysis. Briefly, cells were seeded in 96-well plates in a density of 1×10^4 per well. The cells were acclimatized for 24 hours and subsequently treated with

different concentrations of GO-PEITC, PEITC, and GO (0 – 10 µg/mL) and incubated for 72 hours at 37°C in a CO_2 incubator. The medium was removed after 48 hours and replaced with 100 µL of fresh medium and 5 mg/mL of MTT solution and incubated for another 4 hours under same conditions in a CO_2 incubator. Upon aspiration of medium, purple formazan crystals formed as a result of live cells converting the yellow MTT were dissolved in 200 µL of dimethylsulfoxide (DMSO) to assess the cell viability. The absorbance was read at 540 nm using a microplate reader. All the experiments were conducted in triplicate and the results were expressed as percentage of cell viability with reference to control.

Statistical Analysis

The statistical analysis was done using SPSS 19.0 windows statistical package software, comparing means using One-way ANOVA with Tukey's multiple comparisons test. Statistical significance was checked with regards to p value ($p < 0.05$). All experiments were conducted in triplicates and data expressed as mean ± SEM of three replicates.

RESULTS AND DISCUSSION

The physiochemical determination of the formulated nanocomposite GO-PEITC were done to verify the drug loading using X-ray diffraction (XRD), high resolution transmission electron microscope (HR-TEM), and Infrared Spectroscopic analysis. It was clearly confirmed from the analysis that PEITC was loaded into GO, forming the nanocomposite GO-PEITC (data not given) which is probably done through π-π stacking and hydrogen bond formations. The analysis using high resolution transmission electron microscope also showed a successful conversion of graphite, which usually shows large stacked sheets, to graphene and the formation of nanocomposites GO-PEITC (data not shown). Dynamic light scattering technique was employed to determine the nanocomposite GO-PEITC particle size and was learned that almost 60% of the nanocomposite particles were below 6 nm as revealed by the cumulative distribution frequency. The rest of the GO-PEITC nanocomposites were assumed to have narrow particle sizes ranging from 0-15 nm (data not given). The release of PEITC from the nanocomposite GO-PEITC was determined under human body simulated PBS solution in-vitro and was found that the drug release is sustained under normal physiological conditions (results not given). This proves the ability of GO-PEITC nanocomposite drug formulation to be developed for therapeutic potentials.

The chemopreventive effects of nanocomposite GO-PEITC was determined against human lung cancer (A549), breast cancer (MCF-7), and colon cancer (HT-29) cell lines using MTT

assay. Cell viability is determined through the conversion of MTT yellow compound to non-soluble purple crystal formazan by the mitochondrial dehydrogenases of live cells. The formazan are spectrometrically measured to understand the anti-cancer properties of the formulated nanocomposite GO-PEITC. The results were suggesting that nanocomposite GO-PEITC has chemopreventive effects against human lung cancer (A549), breast cancer (MCF-7), and colon cancer (HT-29) cell lines. It was clear from the results that the nanocomposite GO-PEITC has better anticancer activities on all three cancer cell lines as compared to the pure form of PEITC. The IC_{50} value for nanocomposite GO-PEITC was achieved approximately at 10 µg/mL concentration in both lung cancer (A549) and colon cancer (HT-29) cell lines whereas the IC_{50} value for the nanocomposite was seemingly higher in breast cancer (MCF-7) cell lines. The pure form of PEITC and GO did not achieve IC_{50} in all three cancer cell lines up to the concentration of 10 µg/mL. The formulated drug nanocomposite GO-PEITC might have probably induced apoptosis to the cancer cell lines through various pathways thus suppressing the growth of the cancer cells. Cell viability test of nanocomposite GO-PEITC on normal fibroblast cells (3T3) using MTT assay revealed that the formulated nanocomposite did not exert any cytotoxicity (data not shown) which proves that the nanocomposite does not have toxic effects on normal cells.

Figure caption

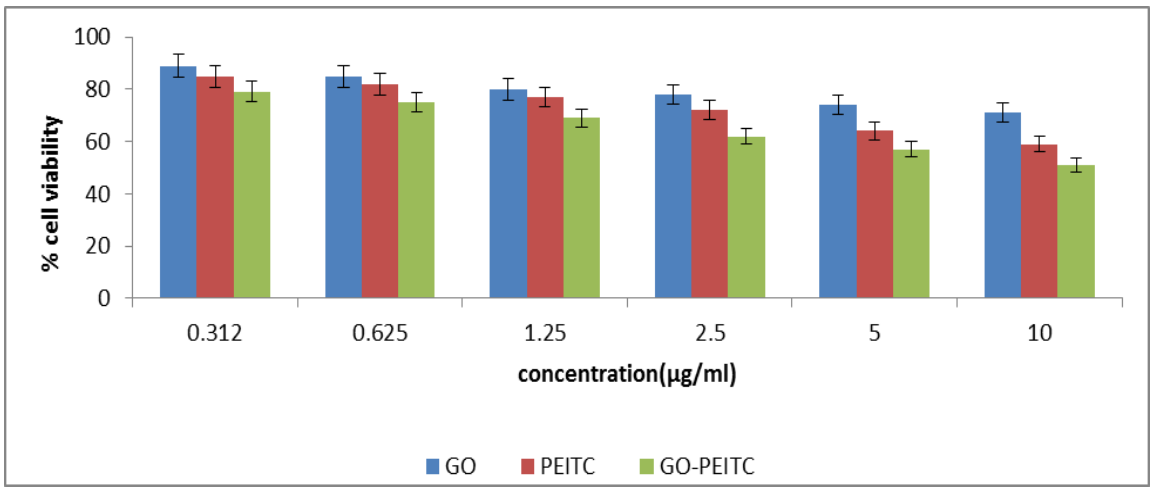

Figure 1(a).

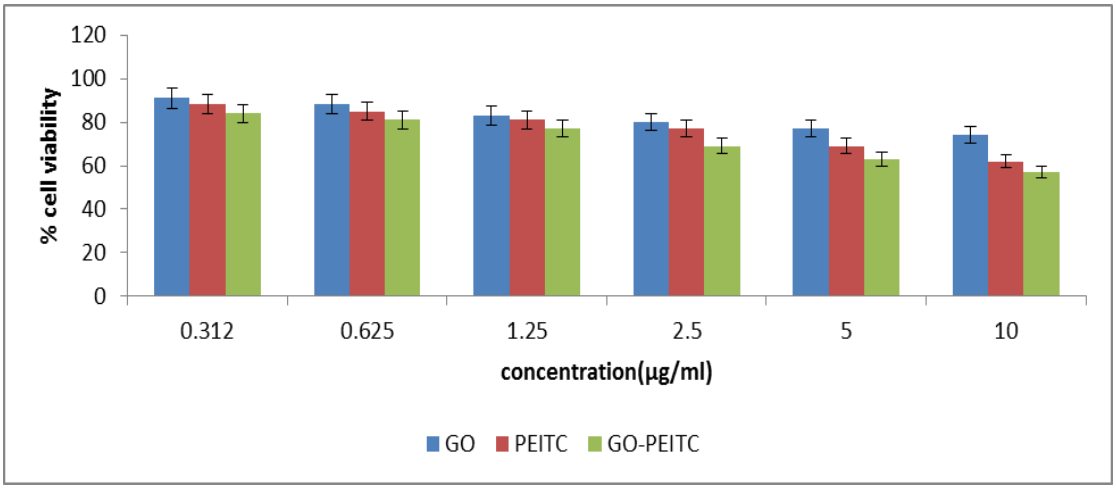

Figure 1(b).

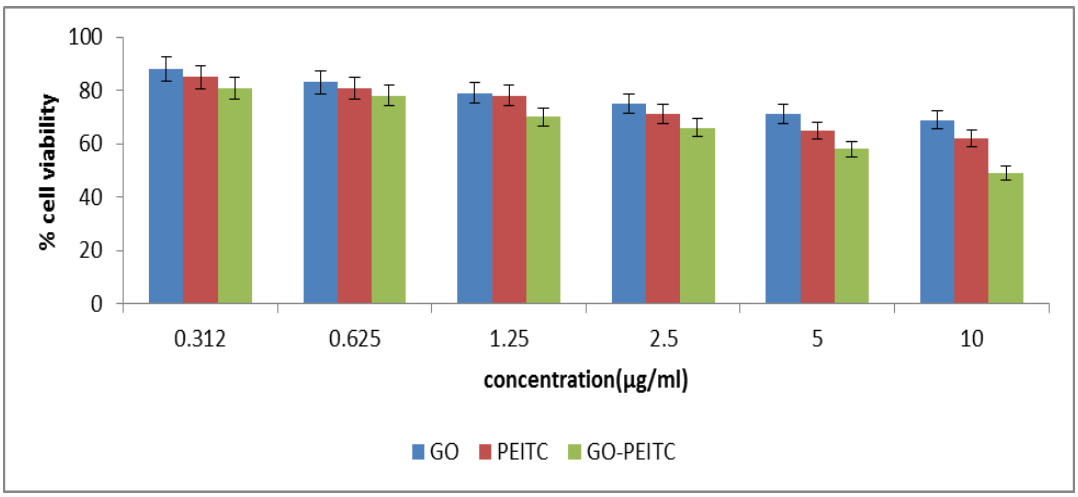

Figure 1(c)

Figure 1: Chemopreventive effects of GO, PEITC and GO-PEITC (0–10 µg/mL) on (a) human lung cancer (A549), (b) breast cancer (MCF-7), and (c) colon cancer (HT-29) cell lines measured by MTT assay. Data represented as mean ± SEM of three independent experiments made in three replicates.

CONCLUSION

From this research, it can be suggested that loading of drug PEITC into GO to form nanocomposites has better chemopreventive effects on human lung cancer (A549), breast cancer (MCF-7), and colon cancer (HT-29) cell lines as compared to the pure form of PEITC. Hence this study can be used as a platform to develop nanocomposites for anticancer treatments. Therefore, it can be concluded that the formulation of nanocomposites have better effects on chemopreventive mechanism in cancer cells.

REFERENCES

1. Ali, M., Husain, Q., Sultana, S. and Ahmad, M. (2018). Immobilization of peroxidase on polypyrrole-cellulose-graphene oxide nanocomposite via non-covalent interactions for the degradation of Reactive Blue 4 dye. Chemosphere, 202: 198-207.
2. Ananya, D. and Vimala, R. (2018). Natural and synthetic polymer for graphene oxide mediated anticancer drug delivery-A comparative study. Int. J. Biol. Macromolec, 107: 2320-2333.
3. Aras, U., Gandhi, Y.A., Masso-Welch, P.A., and Morris, M.E. (2013). Chemopreventive and anti-angiogenic effects of dietary phenethyl isothiocyanate in an N-methyl nitrosourea-induced breast cancer animal model. Biopharmaceutics & Drug Disposition, 34: 98–106.
4. Boreddy, S.R., Pramanik, K.C., and Srivastava, S.K. (2011). Pancreatic tumor suppression by benzyl isothiocyanate is associated with inhibition of PI3K/AKT/FOXO pathway. Clinical Cancer Research, 17: 1784–1795.
5. Dong, F., Dong, X., Zhou, L., Xiao, H., Ho, P.Y., Wong, M.S. and Wang, Y. (2016). Doxorubicin-loaded biodegradable self-assembly zein nanoparticle and its anticancer effect: Preparation, in vitro evaluation, and cellular uptake. Colloids and Surfaces B: Biointerfaces, 140: 324–331.
6. Dorniani, D., Saifullah, B., Barahuie, F., Arulselvan, P., Hussein, M.Z.B., Fakurazi, S., et al. (2016). Graphene oxide-gallic acid nanodelivery system for cancer therapy. Nanoscale Research Letters, 11: 491.
7. Fuentes, I., Silva, P., Martinez, F. and Olea, A.F. (2017). Solubilization of phenol derivatives in polymer micelles formed by cationic block copolymer. Journal of Nanomaterials, 2017; 7187324.
8. Gupta, P. and Srivastava, S.K. (2010). Antitumor activity of phenethyl isothiocyanate in HER2-positive breast cancer models. BMC Medicine, 10: 80.
9. Gupta, P., Adkins, C., Lockman, P. and Srivastava, S. (2013). Metastasis of breast tumor cells to brain is suppressed by phenethyl isothiocyanate in a novel in vivo metastasis model. PLoS One, 2013; 8.
10. Gupta, P., Wright, S.E., Kim, S.H. and Srivastava, S.K. (2014). Phenethyl isothiocyanate: A comprehensive review of anti-cancer mechanisms. Biochimia et Biophysica Acta, 1846(2): 405-424.

11. Harris, R., Lecumberri, E., Mateos-Aparicio, I., Mengíbar, M. and Heras, A. (2011). Chitosan nanoparticles and microspheres for the encapsulation of natural antioxidants extracted from *Ilex paraguariensis*. Carbohydrate Polymers, 84: 803–806.
12. Li, M.F., Liu, Y.G., Liu, S.B., Zeng, G.M., Hu, X.J., et al. (2018). Performance of magnetic graphene oxide/diethylenetriaminepentaacetic acid nanocomposite for the tetracycline and ciprofloxacin adsorption in single and binary systems. Journal of Colloid Interface Sciences, 521: 150-159.
13. Marcano, D.C., Kosynkin, D.V., Berlin, J.M., Sinitskii, A., Sun, Z., Slesarev, A., et al. (2010). Improved synthesis of graphene oxide. ACS Nano, 4: 4806-4814.
14. Meyer-Gerspach, A.C., Wolnerhanssen, B., Beglinger, B., Nessenius, F., Napitupulu, M., Schulte, F.H., Steinert, R.E. and Beglinger, C. (2014). Gastric and intestinal satiation in obese and normal weight healthy people. Physiology & Behavior, 129: 265-271.
15. Mi, L., Hood, B.L., Stewart, N.A., Xiao, Z., Govind, S., Wang, X., et al. (2011). Identification of potential protein targets of isothiocyanates by proteomics. Chemical Research in Toxicology, 24: 1735–1743.
16. Rosas, J.C., Grosso, C.R.F., Aldapa, C.A.G., Vargas, E.R., Marin, M.L.R., Ortiz, F.A.G. and Cortez, R.N.F. (2017). Recent advances in microencapsulation of natural sources of antimicrobial compounds used in food - A review. Food Research International, 102: 575-587.
17. Saw, C.L., Cintron, M., Wu, T.Y., Guo, Y., Huang, Y., Jeong, W.S., and Kong, A.N. (2011). Pharmacodynamics of dietary phytochemical indoles I3C and DIM: Induction of Nrf2-mediated phase II drug metabolizing and antioxidant genes and synergism with isothiocyanates. Biopharmaceutics & Drug Disposition, 32: 289–300.
18. Singh, S.V., Kim, S.H., Sehrawat, A., Arlotti, J.A., Hahm, E.R., Sakao, K., et al. (2012). Biomarkers of phenethyl isothiocyanate-mediated mammary cancer chemoprevention in a clinically relevant mouse model. Journal of the National Cancer Institute, 104: 1228–1239.
19. Subramani, P.A., Panati, K. and Narala, V.R. (2017). Curcumin nanotechnologies and its anticancer activity. Nutrition and Cancer, 69(3): 381-393.
20. Wu, X., Zhu, Y., Yan, H., Liu, B., Li, Y., Zhou, Q. and Xu, K. (2010). Isothiocyanates induce oxidative stress and suppress the metastasis potential of human non-small cell lung cancer cells. BMC Cancer, 10: 269.