CYTOLOGICAL PROPERTIES OF NANO ALLICIN

KELVIN LOUIE D. ANCHETA

An Undergraduate Thesis Submitted to the Faculty of the Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, In Partial Fulfilment of the Requirement For the Degree of

BACHELOR OF SCIENCE IN BIOLOGY
(Major in Zoology)

JUNE 2019

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

APPENDIX TABLES

APPENDIX FIGURES

ABSTRACT

ANCHETA, KELVIN LOUIE D., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **JUNE 2019 CYTOLOGICAL PROPERTIES OF NANO ALLICIN.**

Adviser: CYNTHIA C. DIVINA, Ph.D.
Co-adviser: JUVY J. MONSERATE, Ph.D.

This study was conducted to assess the cytological effect Allicin and Nano allicin using three different bioassays (teratogenicity, cytotoxicity, and genotoxicity). The Nano allicin were synthesized by performing biological synthesis method, which used silver nitrate as a cross linking agent. The successful synthesized of Nano allicin was been validate by the FTIR (Fourier Transform Infrared) analysis.

Based on this study, three different assays were used to examine the possible cytological effects of allicin and nano allicin. Teratogenic effect of allicin and nano allicin were positive in some toxological parameters in lethal effect such as coagulation and no heartbeat also in scoliosis. In addition, Allicin and Nano allicin showed negative results in all of toxicological parameters such as tail and head malformation, growth retardation, etc. Allicin and Nano allicin with 10000 ppm concentration (highest concentration evaluated) showed the highest percent mortality. Hearbeat rate of allicin and nano allicin ranges from 162-180 beats per minute. Hatchability of 100 percent was displayed at allicin concentration of 10 ppm, 1 ppm and the control while nano allicin displayed 100 percent of hatchabilty at concentration of 100 ppm, 10 ppm, 1 ppm and the control. Meanwhile, cytotoxicity testing of allicin and Nano allicin gives off LC50 of 33.107 ppm and 38.010 ppm, respectively, indicating that both allicin and Nano allicin were toxic substance. And lastly, the genotoxicity assay was not determined using *Allium cepa* assay.

LITERATURE CITED

Abiy, E. & Berhe, A., (2016). Anti-Bacterial Effect of Garlic (Allium sativum) against Clinical Isolates of Staphylococcus aureus and Escherichia coli from Patients Attending Hawassa Referral Hospital, Ethiopia. *Journal of Inectious Diseases and Treatment,* 2016, 2:2.

Ankri, S. & Mirelman, D., (1999). Antimicrobial properties of allicin from garlic. *Microbes and infection*, *1*(2), 125-129.

Berthomieu, C., & Hienerwadel, R. (2009). Fourier transform infrared (FTIR) spectroscopy. *Photosynthesis research*, 101, 157-170.

Borlinghaus, J., Albrecht, F., Gruhlke, C.H., Nwachukwu, D., & Slusarenko, A. (2014). Allicin: Chemistry and Biological Properties. *Department of Plant Physiology, RWTH Aachen University, 52056 Aachen, Germany & Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, MB R3T2N2, Canada. Molecules,* 19, 12591-12618.

Busquet, F., Nagel, R., von Landenberg, F., Mueller, S., Huebler, N. and Broschard, T., (2008). Development of a New Screening Assay to Identify Proteratogenic Substances using Zebrafish *Danio rerio* Embryo Combined with an Exogenous Mammalian Metabolic Activation System (m Dar T). *Toxicological Sciences*, *104*(1), 177-188.

Cabuga, C., Abelada, J., Apostado, R., Hernando, B., Lador, J., Obenza, O., Presilda, C., & Havana, H., (2017). *Allium cepa* test; an evaluation of genotoxicity. *Proceedings of the International Academy of Ecology and Environmental Sciences,* 2017, 7(1), 12-19.

Chunlei, P., SengCarlos, L., Semino, P., & McGrath, P., (2004). Zebrafish: a preclinical model for drug screening. *Assay and drug development technologies*, 1(1), 41-48.

Dees, L. (1961). Brine Shrimp. *Fishery Leaflet 527*, 1(1), 2-5

Dockery M., & Tomkins, S. (2000). Brine Shrimp Ecology. *British Ecological Society 2000*, 10 (3), 6-44.

Dulay, R., Kalaw, S., Reyes, R., Alfonso, N., & Eguchi, F. (2012). Teratogenic and Toxic Effects of Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (W.Curt:Fr.) P. Karst. (Higher Basidiomycetes), on Zebrafish Embryo as Model. *International Journal of Medicinal Mushrooms,* 14(5): 507–512.

El Hefny, I., Diab, A., Hozayen, W., & Al-Senosy, N. (2017). Carcinogenic and Cytotoxic Effect of Some Food Additives on *Drosophila melanogaster* and Human Cell Lines.

Department of Genetics, Faculty of Agric., Ain Shams University, Cairo, Egypt. Egyptian Academic Journal Biological Sciences, 9(2): 39- 50.

Fernando, S., Cruz, K., & De Guia, A. (2017). Biologically synthesized gold nanoparticles (AuNP) using pine (*Pinus kesiya*) pollen extract show antifungal activity against *Candida albicans. International Journal of Agricultural Technology*, 13(7.3):2615-2622.

Firbas, P., & Amon, T. (2013). Allium Chromosome Aberration Test for Evaluation Effect of Cleaning Municipal Water with Constructed Wetland (CW) in Sveti Tomaž, *Slovenia. Journal Bioremediation Biodegradation*, 4(4), 1-5.

Halili, J., Ferdin A., & Quilang, J., (2011). The zebrafish embryo toxicity and teratogenicity assay. *The Philippine Biota*, 44, 64-67.

Hamidi, M., Jovanova, B., & Panovska, T. (2014). Toxicological evaluation of the plant products using brine shrimp (*Artemia salina l.*) model. *Macedonian pharmaceutical bulletin*, 60 (1) 9 – 18.

Haroun, H. (2017). Teratogenicity and teratogenic factors. *MOJ Anatomy & Physiology*, 2017, 3(1), 35–39.

Horev, L., Eliav, S., & Izigov N. (2009). Allicin up-regulates cellular glutathione level in vascular endothelial cells. *Europe Journal Nutrition*, 48(2), 67-74.

Koca, I., Tasci, B. & Kutuk, K. (2016). Determination of alliin and allicin in the plant of *Allium scorodoprasum* L. subsp. rotundum by using the infrared spectroscopy technique. *ResearchGate, Acta Horticulture*, 1 (3), 133-140.

Krause, M. (2007). Introduction to Nanotechnology. Veritox, Toxicology and industrial hygiene MSPH, ROH, CIH. Accessed through https://www.aiha.org/aihce07/handouts/rt201krause.pdf.

Kumar, K.P., Bhowmik, D., Chiranjib, B., & Tiwari, P. (2010). *Allium cepa*: A traditional medicinal herb and its health benefits. *Journal of Chemical and Pharmaceutical Research*, 2(1), 283-291.

Lai, K., Shaharuddin, N., Mahmood, M., SYahida, A., & Alafiatayo, A. (2019). Phytochemical evaluation, embryotoxicity, and teratogenic effects of curcuma longa extract on zebrafish (*Danio rerio*). Hindawi; *Evidence-Based Complementary and Alternative Medicine*, 19 (1), 1-10.

Lele, Z. and Krone, P.H., (1996). The zebrafish as a model system in developmental, toxicological and transgenic research. *Biotechnology advances*, *14*(1), 57-72.

Lu, Q., Lu, P.-M., Piao, J.-H., Xu, X.-L., Chen, J., Zhu, L., & Jiang, J.-G. (2014). Preparation and physicochemical characteristics of an alicin nanoliposome and its release behavior. LWT-. *Food Sci. Technol.* (1) 57, 686– 695.

Mansor, N., Herng, H., Samsudin, S., Sufian, S., & Uemura, Y., (2016). Quantification and Characterization of Allicin in Garlic Extract. *Journal of Medical and Bioengineering, 5(1)*.

Martin, A.J., (1992). Cytotoxicity testing in vitro: investigation of 5 miniaturized, colorimetric assays (Doctoral dissertation, Dublin City University). Accessed through http://doras.dcu.ie/18988/.

Martins, N., Petropoulos, S. and Ferreira, I.C., (2016). Chemical composition and bioactive compounds of garlic (*Allium sativum L.*) as affected by pre-and post-harvest conditions: A review. *Food chemistry*, *211*, 41-50.

Majewski, K., Kasika, K., Jakubowski, P., & Podlasz, P. (2017). Influence of fresh garlic (*Allium sativum l.*) Juice on zebrafish (*Danio rerio*) embryos developmental effects. *Journal of Elementology*, 22 (2), 475-486.

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D. and McLaughlin, J., (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica*, *45*(05), 31-34.

Nagel, R., (2002). DarT: the embryo test with the zebrafish *Danio rerio*—a general model in ecotoxicology and toxicology. *Altex*, *19*(Suppl 1), 38-48.

Pancasakti, H., Lunggani, A., & Nurhakkim, M., (2012). Chromosomes and mitotic cell division phase in onion roots after 24 hours acetoorcein soaking time. *BIOMA*,14(2), 46-68.

Pandey, H., Kumar. V., & Roy, B.K. (2014). Assessment of genotoxicity of some common food preservatives using *Allium cepa* L. as a test plant. Laboratory of cytogenetics, *Toxicology reports*, *1*, 300-308.

Patel, R., & Patel, S. (2011). Cytotoxic activity of methanolic extract of *Artocarpus heterophyllus* against A549, Hela and MCF-7 cell lines. *Journal of Applied Pharmaceutical Science* 01 (07), 167-171.

Pirak, T., Jangchud, A., & Jantawat, P. (2012). Characterisation of physical, chemical and antimicrobial properties of allicin–chitosan complexes. *Int. J. Food Sci. Technol.* 47 (7), 1339–1347.

Polat, Z., Sencimen, M., Ozan, U., Ozdem, M., & Ozan, F. (2013). Evaluation of cytotoxic effect of garlic on human gingival fibroblasts: a preliminary study. *Gülhane Tıp Derg*, 55, 276-280.

Qari, S., Ali, K., Mohammed, S., & Sadaqa, E. (2016). genotoxic effect of garlic extract on root tips of *Allium cepa L. IOSR Journal of Pharmacy and Biological Sciences*, 11(2), 41-44.

Rabinkov, A., Miron, T., & Mirelman, D. (2000). S-Allylmercaptoglutathione, the reaction product of allicin with glutathione possesses SH-modifying and antioxidant properties. *Biochim. Biophys. Acta,* 1499(1-2), 144-153.

Romagosa, C., David, E., & Dulay, R, (2016). Embryo-toxic and teratogenic effects of Tinospora cordifolia leaves and bark extracts in Zebrafish (*Danio rerio*) embryos. *Asian Journal of Plant Science and Research*, 2016, 6(2), 37-41.

Rubinstein, L, Mejia, Q., & Parsha, W. (2006). Zebrafish assays for drug toxicity screening. *Expert Opin Drug Metab Toxicology*, 2(2), 231-240.

Saks, M., Upreti, S., & Dang, R. (2017). Genotoxicity: Mechanisms, Testing Guidelines and Methods. *Global Journal of Pharmacy & Pharmaceutical Science* 1(5).

Shah, S. (2012). Importance of genotoxicity and s2a guidelines for genotoxicity testing for pharmaceuticals. *Journal of Pharmacy and Biological Sciences*, 1(5), 43-45.

Shrestha, H. (2004). A Plant Monograph on Onion (*Allium cepa L.*). *The School of Pharmaceutical and Biomedical Sciences Pokhara University, Simalchaur, Pokara*, 7(8), 33-57.

Sudarshan, R., Annigeri, R., & Vijayabala, G. (2012). Garlic and Onion in Dentistry. *Department of Oral Medicine and Radiology, India. IJRAP* 3(4), 556-558.

Ujházy, E., Mach, M., Navarová, J., Brucknerová, I. and Dubovický, M., (2012). Teratology–past, present and future. *Interdisciplinary toxicology,* 5(4), 163-168.

Turlik, I. (2003). The Next Technology Revolution Nanotechnology. Motorola Labs. Accessed through https://www.aps.org/units/fiap/meetings/presentations/upload/turlik.pdf.

Ton, C., Lin, Y., & Willett, C. (2006). Zebrafish as a model for development of Neurotoxicity testing. *Birth Defects Research Part A: Clinical and Molecular Teratology*, *76*(7), 553-567.

Trikić, M., Monk, P., Roehl, H., & Partridge, L. (2011). Regulation of Zebrafish hatching by tetraspanin cd63. *PLoS One*, 6(5): 1-27.

Walker, P. (1952). The mitotic index and interphase processes. *Biophysics Research Unit*, 1(8), 8-15.