

# **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

	PAGE
LIST OF TABLES	vii
LIST OF FIGURES	Ix
LIST OF APPENDICES	x
LIST OF APPENDIX TABLES	xi
LIST OF APPENDIX FIGURES	Xii
ABSTRACT	xiii
INTRODUCTION	1
Background of the Study	1
Objectives of the Study	3
Significance of the Study	4
Scope and Limitation of the Study	5
Time and Place of the Study	5
REVIEW OF RELATED LITERATURE	6
Antimicrobial activity of allicin	6
Antifungal activity of allicin	6
Antiparasitic activity of allicin	6
Antiviral activity of allicin	7
Effects of allicin on animal and human cells	7
Teratogenicity	8
Cytotoxicity	9
Genotoxicity	10
MATERIALS AND METHODS	14
Source of allicin based-material	14
Source of Nano particle	14
Synthesis and characterization of nano allicin	14
Sub-Study I: Teratogenic properties of <i>D. rerio</i>	15
Spawning of zebrafish	15
Collection of fertilized eggs	16
Preparation of treatments and evaluation of zebrafish teratogenicity test	16

Sub-Study II: Cytotoxicity Assay	17
Brine shrimp collection	17
Treatment preparation and evaluation of brine shrimp cytotoxicity test	18
Sub-Study III: Genotoxicity test	19
Plant material and treatment preparation	19
Preparation for maceration and microscopy	20
Statistical Analysis	23
 RESULTS AND DISCUSSION	 24
Synthesis and characterization of nano allicin	24
FTIR Analysis	24
Sub-Study I: Teratogenicity Assay	26
Percentage Mortality	26
Percentage Hatchability	28
Heartbeat Rate	31
Teratogenic Activity of Allicin and Nano allicin	32
Morphological Endpoints of Treated Embryos	34
Sub-Study II: Cytotoxicity Assay	36
Sub-Study III: Genotoxicity Assay	38
Percent Mitotic Index	39
Percent Aberrant Cells	40
Percent Root Growth	41
 SUMMARY, CONCLUSION AND RECOMMENDATION	 43
Summary	43
Conclusion	44
Recommendation	45
 LITERATURE CITED	 46
 APPENDICES	 50

## LIST OF TABLES

TABLE		PAGE
1	Mean percentage mortality of <i>D. rerio</i> embryos after 12, 24, 36, and 48 hours of exposure to different concentrations Allicin	27
2	Mean percentage mortality of <i>D. rerio</i> embryos after 12, 24,36, and 48 hours of exposure to different concentrations Nano Allicin	28
3	Hatchability of embryos treated with different concentrations of Allicin	28
4	Hatchability of embryos treated with different concentrations of Nano allicin	29
5	Heartbeat of <i>D. rerio</i> after 36 and 48 hours of exposure to different concentrations of Allicin	31
6	Heartbeat of <i>D. rerio</i> after 36 and 48 hours of exposure to different concentrations of Nano Allicin	31
7	Lethal and teratogenic effects of various concentrations of Allicin at 12, 24, 36, and 48 hours of exposure	34
8	Lethal and teratogenic effects of various concentrations of Nano Allicin at 12, 24, 36, and 48 hours of exposure	35
9	Mean percentage mortality of <i>A. salina</i> nuplii after 24 hours of exposure to different concentrations of Allicin	36
10	Mean percentage mortality of <i>A. salina</i> nuplii after 24 hours of exposure to different concentrations of Nano allicin	37
11	Mean percentage mitotic index of <i>Allium cepa</i> test after 3 days' exposure to different treatment concentration of Allicin	38
12	Mean percentage mitotic index of <i>Allium cepa</i> test after 3 days' exposure to different treatment concentration of Nano allicin	39
13	Mean percent aberrant cells of <i>Allium cepa</i> test after 3 days' exposure to different treatment concentration of Allicin	40
14	Mean percent aberrant cells of <i>Allium cepa</i> test after 3 days' exposure to different treatment concentration of Nano allicin	41

15	Mean percent root growth of <i>Allium cepa</i> after 3 days' exposure to different treatment concentration of Allicin	42
16	Mean percent root growth of <i>Allium cepa</i> after 3 days' exposure to different treatment concentration of Nano allicin	42

## LIST OF FIGURES

FIGURE		PAGE
1	FTIR Spectral wavelength of Allicin	24
2	FTIR Spectral wavelength of Nano allicin	25
3	Gross morphology of <i>D. rerio</i> embryos treated with Allicin at 48 hpta	33
4	Gross morphology of <i>D. rerio</i> embryos treated with Nano Allicin at 48 hpta	33

## LIST OF APPENDICES

APPENDIX		PAGE
A	ANOVA Tables of the Results of Teratogenicity and Cytotoxicity Assay	51
B	Probit analysis of Cytotoxicity	53
C	Comparison of allicin and nano allicin using t-test	56
D	Probit Analysis Graph	58
E	Experimental Procedure	60

## APPENDIX TABLES

APPENDIX TABLE	PAGE
1 Analysis of variance of mean heart beat rate of zebrafish embryo treated with Allicin after 36 hours of exposure	51
2 Analysis of variance of mean heartbeat rate of zebrafish embryo treated with Nano Allicin after 36 hours of exposure	51
3 Analysis of variance of percentage mortality of <i>A. salina</i> nauplii treated with Allicin after 24 hours of exposure	51
4 Analysis of variance of percentage mortality of <i>A. salina</i> nauplii treated with Nano Allicin after 24 hours of exposure	52
5 Probit Analysis parameters estimates for treatment concentration of Allicin	53
6 Probit Analysis parameters estimates for treatment concentration of Nano Allicin	53
7 Probit analysis for the median lethal concentration (LC50) of treatment concentrations of Allicin	53
8 Probit analysis for the median lethal concentration (LC50) of treatment concentrations of Nano Allicin	54
9 Comparison of allicin and nano allicin using t-test (mortality)	56
10 Comparison of allicin and nano allicin using t-test (hatchability)	56
11 Comparison of allicin and nano allicin using t-test (heartbeat)	57
12 Comparison of allicin and nano allicin using t-test (cytotoxicity)	57

## APPENDIX FIGURES

APPENDIX FIGURES	PAGE
1 Point estimate of LC50 value of Allicin after 24 hours of exposure	58
2 Point estimate of LC50 value of Nano c Allicin after 24 hours of exposure	59
3 Materials used in synthesized of Nano allicin	60
4 Process of synthesis of Nano allicin	60
5 Magnetic stirring of allicin solution and the result of synthesized nano allicin	61
6 FTIR Analysis	61
7 Zebrafish acclimatization	62
8 Collection of Zebrafish fertilized eggs	62
9 Observation of zebrafish teratogenicity test	63
10 Collection of brine shrimp	63
11 Brine Shrimp putting on vials	64
12 Cytotoxicity set-up	64
13 Cytotoxicity observation	65
14 Genotoxicity set-up	65
15 Observed root in control (genotoxicity)	66
16 Serial Dilution set-up	66

## 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 Infectious 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-Senousy, 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., Heng, H., Samsudin, S., Sufian, S., & Uemura, Y., (2016). Quantification and Characterization of Alicin 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 alicin–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-Allylmercaptogluthathione, 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, Pokhara*, 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.