*Available online at* www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2293

**ISSN: 2320 – 7051**

*Int. J. Pure App. Biosci.* **4 (3):** 16-20 (2016)

International Journal of Pure & Applied Bioscience

*Research Article*

OPEN ACCESS

# Embryo-Toxicity and Teratogenicity of *Derris elliptica* Leaf Extract on Zebra Fish (*Danio rerio*) Embryos

**Josephine Joy V. Tolentino[1]* and Jerwin R. Undan[2]**

[1] Graduate Student, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, 3120 Philippines

[2] Molecular Biology and Biotechnology Laboratory, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, 3120 Philippines

*Corresponding Author E-mail: jjvtolentino0126@gmail.com

Received: 19.05.2016 | Revised: 2.06.2016 | Accepted: 7.06.2016

**ABSTRACT**

*The toxic effects of Derris eliptica or "Opay" were assessed against zebrafish embryos. Leaf extract of D. elliptica via hot water extraction and the zebra fish (Danio rerio) as the model organism were utilized for the assessment of its teratogenicity and embryo-toxicity potential. Two concentrations of 0.05% ($T_2$) and 0.50% ($T_3$) of leaf extract were used, while embryo water medium was used as the control ($T_1$). Percent mortality was observed after 12, 24, and 48 hours. In addition, percent hatchability was observed after 48 hours only. The number of heart beats per minute of the zebra fish embryo within 48 hours post treatment was also recorded. The results showed that zebra fish embryos treated with 0.05% extract showed reduced hatchability rate, lower heartbeat rate and delayed formation compared with the control. The adverse effect of 0.50% treatment was still severe resulting in undeveloped head and tail region, coagulation and death of embryos. It was concluded that teratogenic and lethal effects on zebra fish increased with the increase in the dose concentration of leaf extracts of D. elliptica. Further investigation on this plant species affecting other animals with ethanol and acetone extracts are also recommended.*

***Key words:*** *Teratogenic, Embryo, Zebra fish, Derris elliptica, Hot water leaf extract*

## INTRODUCTION

Some plants are known to possess piscicidal activities or fish poisons. Such plants are used by some communities for fishing. In an indigenous community of Ikalahan tribe, Sta. Fe, Nueva Vizcaya, Philippines, a known wild type of plant, *Derris elliptica* or "Opay" in their dialect, is commonly used as a fish poison, since it has been known that the plant leaves had toxic effects to fishes. *D. elliptica* belongs to the family Fabaceae characterized as a large climber which is cultivated mainly for its roots as a source of insecticide (Rotenone) in some tropical countries[1]. Some of the potential uses of piscicides are for cultural, commercial and environmental reasons; though most countries prohibit the use of piscicides in large scale killing of fish, but for some countries like Africa, South America, Philippines and areas in South Pacific, fish poisons are used to catch fish for food[2].

**Cite this article:** Tolentino, J.J.V. and Undan, J.R., Embryo-Toxicity and Teratogenicity of *Derris elliptica* Leaf Extract on Zebra Fish (*Danio rerio*) Embryos, *Int. J. Pure App. Biosci.* **4(3):** 16-20 (2016). doi: http://dx.doi.org/10.18782/2320-7051.2293

The present study intends to assess the gross morphological endpoints of zebrafish and determine any abnormalities as affected by different concentrations of *D. elliptica* leaf extract. Establishment of this plant's leaf extract concentration that can possess teratogenic and lethal effects on zebrafish is also aimed in this study.

## MATERIALS AND METHODS

### Preparation of Plant Leaf Extract

The collected leaves of *D. elliptica* from Imugan, Sta. Fe, Nueva Vizcaya were sun-dried, shredded into smaller pieces and were further finely crushed until powdery using sterile blender. 20g of powdered leaves was placed in a sterile flask with 200 mL of distilled water and was evenly stirred. The solution was then subjected into a hot water bath maintained at 80°C for 2 hours and filtered.

### Spawning of Mature Zebrafish

A glass aquarium filled with untreated, clean tap water with continuous supply of oxygen was provided to house adult male and female zebra fishes in 1:2 ratio based after a protocol[3]. To instigate spawning of zebra fishes, the glass aquarium was covered with a black plastic sheet (dark condition) for 12 hours. Once spawning was finished, the eggs were subjected to a fluorescent bulb (lighted condition) for 12 hours as well. Fertilization of eggs takes place within 20 minutes after the introduction of light (Nagel, 1998). The fertilized eggs were siphoned out of the aquarium after 12 hours of fertilization using a hose. The embryos were then rinsed three times and placed in a clean watch glass containing embryo medium for microscopic observations.

### Establishing Teratogenic assay

*D. elliptica* hot water extract was diluted using embryo water. Two concentrations were prepared separately as 0.05% ($T_2$) and 0.5% ($T_3$). Pure embryo water was used to serve as control set-up and was labeled as 0% ($T_1$). $T_2$ has 500 µL of leaf extract mixed with 9.995 mL of embryo water; while $T_3$ has 50 µL of leaf extract in 9.95 mL of embryo water, respectively.

### Teratogenicity and Embryo toxicity of *D. elliptica* Extract

After the complete examination of normal embryos, 4 ml of each treatment concentration was doled out into a 12-well ELISA plate using a micropipette. Three fertilized embryos were placed into each well per replicate using a sterile plastic dropper. Plates were incubated at 26 ± 1°C.

### Microscopic Observation and Analyses

The fertilized embryos were observed first under a light microscope before placing in treatments. For each of the treatments, the teratogenic activity of the *D. elliptica* plant extract was examined through the aid of a compound microscope after 12, 24, and 48 hours after the incubation period. Evaluation of toxicological and morphological endpoint of zebrafishes was based on parameters established by Nagel (1994) as*: teratogenic* (malformation of head, malformation of tail, growth retardation, scoliosis/flexure, stunted tail, and limited movement); *lethal* (coagulation, tail not detached, no somites and no heartbeat); and *normal.*

### Presentation of Data and evaluation of mortality and hatchability rates of zebrafish

The data recorded were used to (a) assess gross morphological endpoints of zebrafishes; (b) determine *Percent mortality* (characterized by the number of coagulated zebrafish embryos after 12, 24, and 48 hours post-treatment application (hpta)) computed using the formula:

$$\%\, Mortality = \frac{No.\ of\ dead\ embryos}{Initial\ no.\ of\ embryos}\ x\ 100$$

(c) Percent hatchability (characterized by hatched zebrafish embryo after 48 hpta) computed using the formula:

$$\%\, Hatchability = \frac{No.of\ hatched\ \ embryos}{Initial\ no.of\ embryos}\ x\ 100$$

(d) Number of heart beats per minute of zebrafish embryo in different treatment concentrations within 48 hpta was counted and averaged.

### Statistical Analyses

Three replicates of each treatment were laid out in a Complete Randomized Design (CRD) and were analyzed in One-Way Analysis of Variance (ANOVA). Significant treatment comparison at 5% level of significance was determined using Duncan Multiple Range Test (DMRT), LSD and Tukey's Test using the SPSS version 21.0 program.

## RESULTS

Among the treatment groups, $T_1$ (control) has normal development of embryos. At 48 hpta, hatching was completed for the two treatment concentrations ($T_1$ and $T_2$). The percent hatchability of embryos treated with $T_2$ (0.05%) and $T_3$ (0.5%)were significantly lower than that of the control after 48 hpta (Fig.1). There were no recorded hatchlings for 0.5% ($T_3$) because after 48 hpta, all embryos under this treatment group died. The highest number of heartbeat per minute was recorded in embryos incubated with embryo water (control) with a mean of 169 beats per minute (bpm). This was not significantly different from treatment 2 but with lower heartbeat rates of162 bpm. In contrast, no heartbeats were recorded on the treatment 3 due to death of embryos (Fig.2).

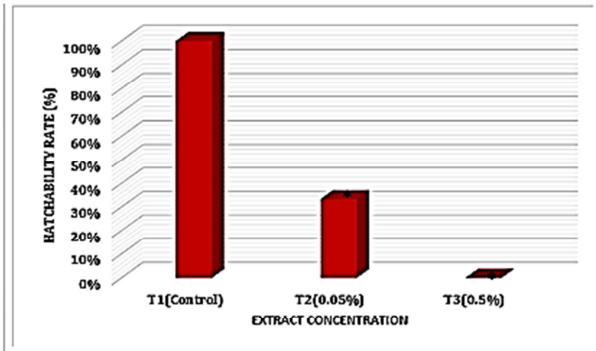


**Fig.1. Percent hatchability of the embryos 48 hpta in different treatment concentrations. The hatchability rate of $T_1$ (control) is significantly higher than $T_2$ and $T_3$. * represents a significant difference to the control.**

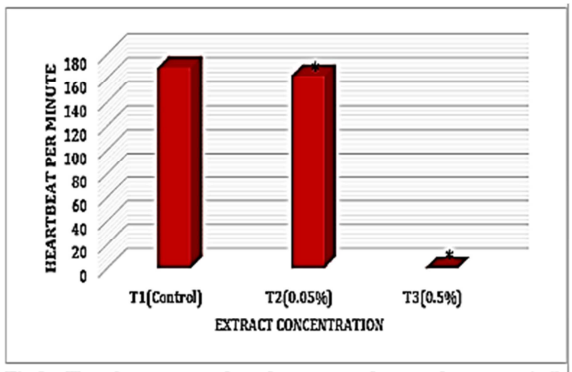


**Fig.2. Heartbeat rate of embryos at pharyngula stage (tail pigmentation) in different treatment concentrations after 48 hpta. Lower heartbeat rates (162bpm) was recorded in $T_2$ as compared to $T_1$ (169bpm). No heartbeats recorded in $T_3$ due to death of embryos.**

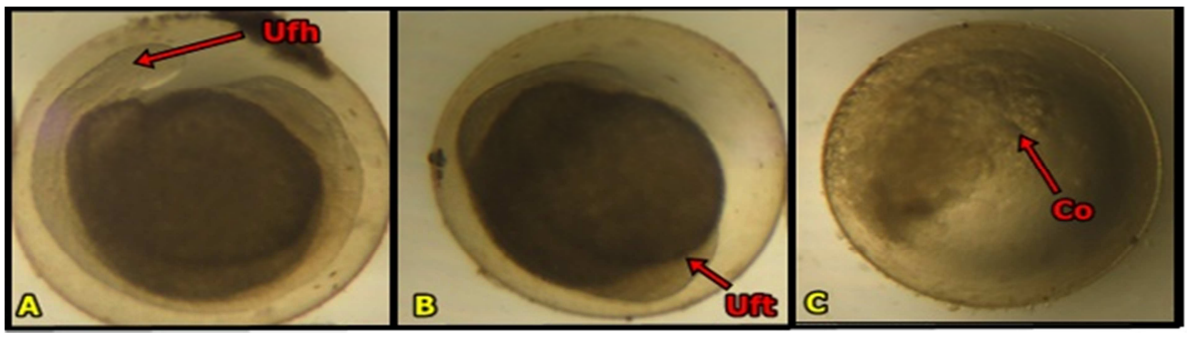


**Fig.3. Lethal and teratogenic effects of *D. elliptica* on zebrafish embryos. (A-B) Unformed head and tail regions were observed in embryos exposed to $T_3$ (0.5%) after 24 hpta. (D) Coagulation and no visual heartbeat leading to subsequent death of the embryo was observed after 48 hpta in $T_3$ (0.5%). Ufh- unformed tail; Uft- unformed head; and Co- coagulated.**

Different toxicological and morphological abnormalities in zebrafish embryos caused by *D. elliptica* leaf extract were noted as unformed head, unformed tail, coagulation and death for those exposed to 0.5% treatment concentrations of the extract (Fig.3 A-C). On the other hand, embryos exposed to $T_2$ (0.05%) showed growth retardation and limited movements as to compare with the control group. Mortality rates of zebrafish embryos after subsequent exposure to different extract concentrations led to early death of embryos specifically in $T_3$ (0.5%). The mortality rates of embryos treated with $T_3$ (0.5%) are significantly higher than $T_2$ (0.05%) and control treatment groups respectively (Fig.4). No visual heartbeat and coagulation of the embryos were observed after 12 and 48 hpta of $T_3$ (0.5%) of the leaf extract. All embryos of zebrafish were dead after 48 hours of post-treatment exposure of $T_3$ (0.5%) of *D. elliptica* leaf extract.

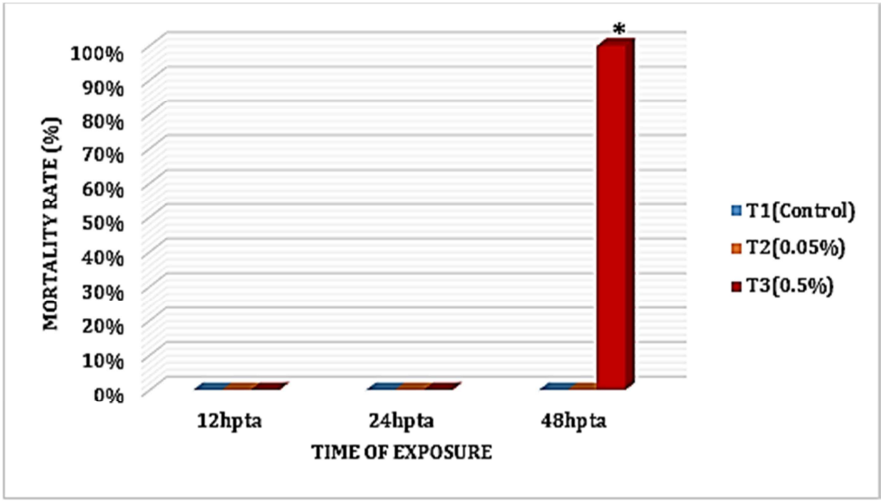


**Fig.4. Percent mortality of embryos after 12, 24 and 48 hours of treatment exposure to *D. elliptica.* Embryos treated with 0.5% extract concentration showed death of all embryos after 48hpta; which is significantly higher than the other two treatment groups. * means significant difference.**

## DISCUSSION

This study was able to test the embryo-toxic and teratogenic effects of the *D. elliptica* leaf extract against zebrafish as a model organism which could also be toxic for the development and growth of other vertebrate embryos which could be in a resemblance of effects among zebrafish embryos since the development of embryo in zebrafish is very similar with higher vertebrates[4]. Teratology is the aspect of embryology involved with the study of abnormal development and a teratogen is any agent which overtly causes the production of a congenital defect or increases incidence of a particular congenital defect[5]. *D. elliptica*, being a teratogen, was assessed for the extent of its teratogenic and lethal abilities wherein varying concentrations of the leaf extract affected the hatchability and even the rate of mortality on the zebrafish embryos. Apart from leaf extract, the aqueous root extract of *D. elliptica* was also found to be toxic to golden snail (*Pomacea spp.*) at 2000 ppm while the stem gave only 30% mortality at 10,000 ppm at the rate of 90kg/ha; and hence can be used as an effective snail control[6].

Zebrafish embryos that were exposed to lower concentration (0.05%) of *D. elliptica* leaf extract exhibited growth retardation due to limited number of hatchlings produced after 48 hpta in contrast with the hatchability rate of embryos observed under normal embryo water medium (control group). The increased concentration to 0.05% severely affected the hatchlings and caused early death of embryos after 48 hpta. Thus, an inverse relationship between percent hatchability and concentration of the leaf extract could be perceived; where the increase in amount of concentration of the extract causes decrease in hatchability rate of the zebrafish embryos. Using one-way analysis of variance, there is statistical significant difference on the percent hatchability among the treatment samples. Low hatchability rate could be attributed to the delayed development of zebrafish embryos, and can therefore be one of the important aspects of the sub-lethal properties of the plant extract[4].

Embryos exposed to $T_3$ (0.5%) extract concentration of the *D. elliptica* plant showed malformation (underdevelopment) of the head and tail regions, growth retardation, limited movement and coagulation after 24 hours of post-treatment application. The failure of organogenesis leads to the underdevelopment of the head and tail morphologies; which can be ascribed to the inhibition or disturbance of vital compounds essential for growth and development of embryos[4].

The zebrafish embryos exposed to teratogen plant extract, which in this study was *D. eliptica* caused developmental delay, growth retardation and even early death of the embryos. In this present study, the embryos treated with 0.5% concentrations of the extract were characterized with delayed growth and

development prior to malformations and early death of the embryos which was statistically significant using one-way analysis of variance. Further, the morphological endpoints related to the delayed development of zebrafish embryos can be caused by the presence of boric acid (for teratogenic concentrations) and valproic acid as well as methoxyacetic acid (for non-teratogenic concentrations[7]. No heartbeat rate was recorded in $T_3$ (0.5%) concentration among all its replicates due to the early death of the embryos.

This study was able to reveal that in just very minimal concentrations of *D. elliptica* such as 0.5% can be very toxic to fish embryos. Even at low concentrations, congenital deformities and malformations were observed and prolonged exposure to the leaf extract of this plant can be fatal to the embryos. Further investigation on this plant species affecting other vertebrates using ethanol and acetone extracts are also suggested for future studies.

## ACKNOWLEDGEMENT

The authors thank DOST ASTHRDP SEI for providing financial assistance on this research study and reviewers of this article for their helpful comments and suggestions.

## REFERENCES

1. Starr, F., Starr, K. and Loope, L. *Derris elliptica*. United States Geological Survey—Biological Resources Division Halekeala Field Station, Maui, Hawaii. (2003) 1-4.
2. Burton, R., Cannon, J., Owen, N., and Wood, S. *Jour. of Chem. Edu.* **81(10):** 1457-1461 (2004).
3. Nagel, R., In W. Heger, S. Jung, S. Martin. UBA Texteband Berlin: Umweltbundesamt. 80-93 (1998).
4. Dulay, R., Kalaw, S., Reyes, R., and Cabrera, E. *Ann. of Biol. Res.***5 (6):** 9-14(2014).
5. Moore, K.S. 2013. The Developing Human. 9th edition. Saunders Elsevier, Inc. Canada.13-21.
6. Maini, P. and Morallo-Rejesus,R. *Phil. Jour. of Sci.***64:** 21-29 (1993).
7. Teixido, E., Pique, E.; Gomez-Catalan, J., Llobet, JM. *Toxicol in Vitro,* **27:** 469-478 (2013).