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Toxic and Teratogenic Effects of Water Leaf Extract of *Momordica charantia* in Zebrafish (*Danio rerio*) Embryos

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ABSTRACT

This paper reported the toxic and teratogenic effects of water leaf extract of Momordica charantia in developing embryos of Danio rerio. The mortality, hatchability, delayed development and morphological abnormalities of extract treated embryos were determined. After 36 and 48 h of exposure, 100% mortality was observed in embryos at 3% of extract. The toxic effects of the extract were dependent on extract concentration and time of exposure. Embryos exposed to 0.1%, or higher concentrations showed delayed development. Control embryos showed 100% hatchability while no hatching was observed in embryo exposed at 0.1% or higher concentrations. Treated embryos had bent back, scoliosis, edema in the yolk sac, bent tail tip as manifestations of teratogenic effects of the plant extract.

Keywords: *Momordica charantia*, *Danio rerio*, Teratogenicity, Cytotoxicity, Abnormality

INTRODUCTION

Many plants are used by humans as source of food, shelter, fiber, and even medicine. They are indispensable source of safe and natural agents in both for traditional and modern medicine due to their several bioactive chemical components. Nowadays, many drugs available in the market have their origin from the herbal medicinal plants which are discovered from the different cultures. In the Philippines, *ampalaya* is one of such plants that have been utilized in alternative medicine especially in diabetes treatment.

Momordica charantia, commonly known as *ampalaya* by Filipinos, is a flowering vine belonging to the family Cucurbitaceae, with serrated leaves and bears yellow flowers. Its oblong and wrinkled fruit when young is emerald green that turns to orange-yellow when ripe. All parts of the plant, including the fruit have bitter taste. In a review by Haque et al. [1], this plant is a traditional medicine that has abortifacient, antimutagenic, antimycobacterial, antidiabetic, anthelmintic, antimalarial, antiviral, antitumor, antileukemic, antibacterial, antioxidant, antiulcer, anti-inflammatory activities along with hypo-cholesterolemic, hypo-triglyceridemic, hypo-tensive, immunostimulant, and insecticidal properties. It is also used in treating colds and fevers, constipation in children, gastroenteritis, stomach aches and some viral infections [2]. Moreover, *M. charantia* have shown its efficacy on various cancer cell lines like choriocarcinoma, melanoma, breast cancer, lymphoid leukemia, lymphoma, skin tumor, and prostate cancer, squamous carcinoma of tongue and larynx, human bladder carcinomas and on Hodgkin's disease [1].

Danio rerio (zebrafish) assay is a well-accepted test system for toxicology and teratogenic studies [3]. Due to the transparency and fast development of its embryo, cost efficient, and any malformation or abnormal development attributed to the treatment can monitored easily. Thus, it is an effective animal model used to indicate the presence of potential carcinogens, mutagenic and teratogenic compounds or substances.

Several studies have been reported on the pharmacological, medicinal, and folkloric uses of *M. charantia*. However, the teratogenic and embryonic effects on zebrafish have not been studied in detail, thus, this study focused on the effects of the water leaf extract in the developing embryos of zebrafish.

MATERIALS AND METHODS

Source of plant samples

The leaves of *Momordica charantia* were collected from Science City of Munoz, Nueva Ecija, Philippines and were brought in the laboratory. Leaves were washed three times and air-dried for 7 days. These were pulverized and subjected to extraction.

Extraction and treatment preparation

The extract of *M. charantia* was obtained following the hot water extraction protocol of Eguchi et al. [4]. Sample (10 g) was extracted in 300 ml hot water at 80-90°C in a water bath for 2 h. After which, the extract was filtered using Whatman filter paper No. 2. Ten ml of each treatment concentration of the extracts was prepared using embryo water as diluent (0.05%, 0.1%, 0.5%, 1% and 3%) and control (embryo water) and placed into each well of the 12-well ELISA plate for toxicity assay.

Spawning and fertilization

The procedures on spawning and fertilization were followed after Nagel [3]. Mature female and male zebrafish at 1:2 ratios were acclimatized in a glass aquarium with water saturated with oxygen. They were fed two times a day with dry flakes and the quality of water was maintained. In spawning, fish were localized in a plastic mesh and the aquarium was covered with black plastic sheet for 12 h. After spawning, eggs were exposed to lighted condition for another 12 h. The eggs were fertilized after 30 min of exposure to light. Embryos were collected from the aquarium using a hose and examined for the uniformity of embryos using a microscope. Coagulated and unfertilized eggs were discarded and the normal embryos were used in the assay.

Toxicity and teratogenicity assay

The protocol on the toxicity and teratogenicity using zebrafish embryos was adopted from Dulay et al. [5]. Four embryos at segmentation phase were exposed to the different concentrations of the extract. Mortality was determined after 12, 24, 36 and 48 h of extract exposure. The hatching rate and heartbeat rate were also recorded. The morphological abnormalities of the treated embryos were based on the parameters established by Nagel [3]. The validity of the results was also noted. Analysis of Variance (ANOVA) was used to analyzed the data and Least Significant Difference (LSD) was used to compare the means at 5% level of significance.

RESULTS AND DISCUSSION

Toxic Effect of *Momordica charantia* extracts to *Danio rerio* embryos

Mortality is the number of deaths that occur at a specific time due to the toxic effects of a substance. The toxicity assay confirmed the toxic effect of *Momordica charantia* on zebrafish embryos. The percentage mortality of *Danio rerio* embryos after 12, 24, 36 and 48 h of exposure in varying concentrations of water leaf extract of *M. charantia* is presented in Table 1. Apparently, the mortality is more evident with increasing concentration of the extract. A 100% mortality of embryos was observed at 3% after 36 and 48 h. Embryos at 0.1% which showed no mortality after 36 h had 8.33% mortality at 48 h exposure whereas embryos at 0.5% with consistent 8.33% mortality from 12 h to 36 h recorded 16.67% at the last observation period. These observations confirmed the concentration and time exposure dependency of the toxic effects of *M. charantia* extract. However, embryos exposed at 0.05% of extract showed no mortality in all observation periods. This toxic effect obtained in the present study conforms to the cytogenotoxic effects of *M. charantia* water extract in meristematic onion root tip [6]. Moreover, leaf extract of this plant also showed toxic effect against larvae and pupa of *A. stephensi* [7] and abortifacient activity during pregnancy in lower and higher forms of organisms [1]. The fruit and seeds extracts of *M. charantia* demonstrated greater toxicity than its leaf extract [8].

Table 1: Mortality of *D. rerio* embryos after 12, 24, 36, and 48 h of exposure to varying concentrations of *Momordica charantia* aqueous leaf extract

Concentration (%)	Mortality (%)			
	12 h	24 h	36 h	48 h
Embryo water	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}
0.05	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}
0.1	0.00^{b}	0.00^{c}	0.00^{c}	8.33^{cd}
0.5	8.33^{b}	8.33^{bc}	8.33^{bc}	16.67^{bc}
1.0	16.67^{a}	16.67^{b}	16.67^{b}	33.33^{b}
3.0	33.33^{a}	58.33^{a}	100.00^{a}	100.00^{a}

In column, treatment means with the same letter of superscript are significantly different from each other using DMRT at 5% level of significance

Plant extract at different concentrations may exhibit various cytotoxicity manifestations. This toxic effect could kill cancer cell in the cultured cell, eliminate pest, and can be attributed to a wide range of pharmacologic effects [9]. For instance, extract of *Solanum nigrum* demonstrated cytotoxic potential against HeLa cells and in Vero cell, indicating the use of this plant for anti-cervical cancer treatments [10]. Moreover, *Achyrocline satureioides*, *Aristolochia macroura*, *Lithraea molleoides* and *Schinus molle* have showed potent inhibitory properties against human hepatocellular carcinoma cell line [11].

Effect of *Momordica charantia* leaf extract on the hatchability of embryos

On the progression of hatching, as described by Kimmel et al. [12], the following development occurs; completion of rapid morphogenesis of primary organ systems; cartilage development in head and pectoral tin; hatching occurs asynchronously. The effect of *M. charantia* leaf extract on the hatchability of exposed embryos was determined and the results were presented in Table 2. Apparently, the varying concentrations of the extract affected the hatchability of embryos, which is inversely proportional: as the extract concentration increases, the percent hatchability decreased. Control embryos showed 100% hatchability while 0.05% concentration had 50% hatchability. However, no hatching was observed in embryo exposed at 0.1% or higher concentrations of *M. charantia* leaf extract. The results strongly suggest that the extract interrupted the normal hatching process of the zebrafish. This could be due to the delayed development and morphological abnormalities of the embryo. This effect of *M. charantia* on the hatching process obtained in the present study was also observed in mosquito eggs when treated with ethyl acetate extract of *M. charantia* leaf [7].

Table 2: Hatchability of *Danio rerio* after 36 h of exposure to varying concentrations of *Momordica charantia* aqueous leaf extract

Concentration (%)	Hatchability (%)	Delayed development(%)
Embryo water	100.00[a]	0.00[c]
0.05	50.00[b]	50.00[b]
0.1	0.00[c]	100.00[a]
0.5	0.00[c]	100.00[a]
1.00	0.00[c]	100.00[a]
3.00	0.00[c]	100.00[a]

In column, treatment means with the same letter of superscript are significantly different from each other using DMRT at 5% level of significance

Delayed development of embryos treated with *Momordica charantia* leaf extract

One of the most distinct teratogenic effects of *M. charantia* leaf extract was the delayed development. A 100% delayed development was noted in 0.1% or higher concentrations. However, 0.05% had 50% of delayed embryos. No delayed development was observed in control embryos and this obvious delayed development of embryos is due to the different concentrations of extract is shown in Figure 1. Same observation was presented in the study of Ononuju and Nzenwa [13] where *M. charantia* extract slows down the growth progress of nematode's egg. The delayed growth could be accounted to the abortifacient properties and other active components of *M. charantia* such as beta-momorcharin. The abortifacient activity of beta-momorcharin is seen via (a) blockage of the hatching of embryos from the zonapellucida; (b) decrease in attachment of the blastocyst; (c) reduction in the trophoblast outgrowth and (d) disruption of inner cell mass development [14].

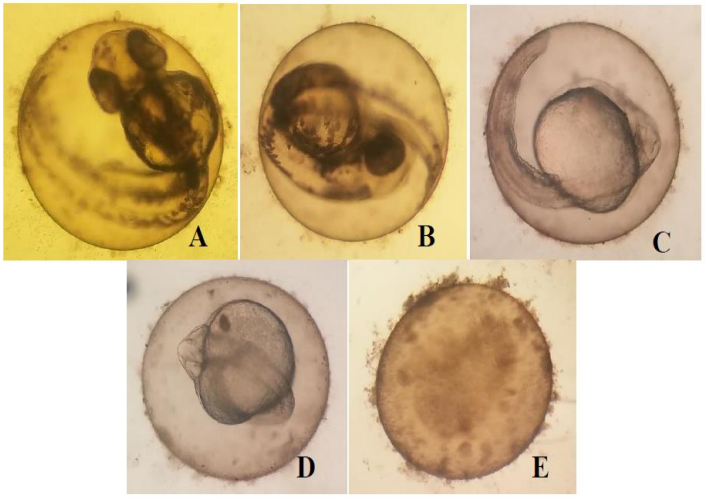

Figure 1: The development of *Danio rerio* embryos at varying concentrations of water leaf extract of *Momordica charantia*; A) 0.05%, B) 0.1%, C) 0.5%, D) 1%, E) 3% after 36 h of exposure

Teratogenic effect of *Momordica charantia* leaf extract

The different morphological abnormalities of embryos treated with varying concentration of *M. charantia* extract were also observed after 72 h of extract exposure. The different morphological endpoints are shown in Figure 2. The *M. charantia* extract caused embryos with bent back embryo at 1% concentration and embryos with scoliosis, edema in the yolk sac, bent tail tip at 0.5% concentration. Consequently, this medicinal plant could exhibit teratogenic effects in the developing embryos of *D. rerio*. In previous work, aqueous leaf extract of *M. charantia* induced an increase in the production of sperms of male albino rats with abnormally shaped heads as the dosage increases which results invariability of the sperm cells [15]. Moreover, Fernandopulle et al. [16] reported that the unripe fruit of *M. charantia* showed teratogenic effects in rats having external malformation and dwarfed at 10%, while the 20% showed skeletal abnormalities. Other plants also exhibit teratogenic effects in mammalian model. Oral administration of *Pandanus conoideus* fruit extract to pregnant *Rattus norvegicus* caused lordosis skeleton to the developing fetuses [17]. Potential teratogenicity of the root and bark ethanolic extracts of *Rauwolfia vomitoria* are both teratogenic that caused cerebral tissue damage to the fetuses of albino wistar rat [18].

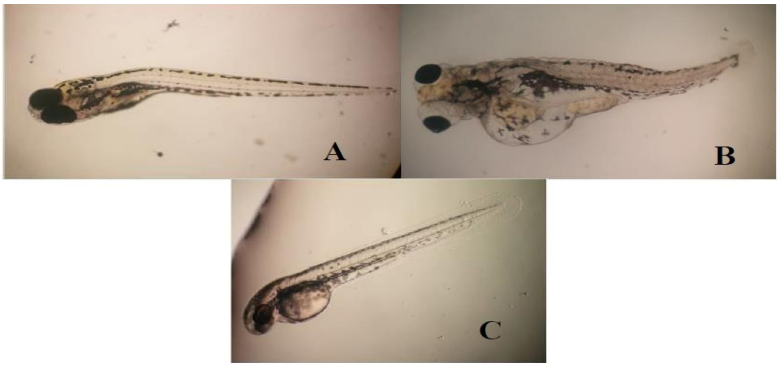

Figure 2: Teratogenic effects of varying concentrations of *Momordica charantia*extract against *Danio rerio* embryos: A) Bent back at 1% concentration B) scoliosis, edema in the yolk sac, bent tail tip at 0.5% concentration, and C) normal control embryo

Based on the collective findings in this study, it proves that the *M. charantia* water leaf extract exhibited toxic and teratogenic effects to the developing embryos of *D. rerio*. The survival, normal development, and hatchability were affected by the extract in dose and time exposure dependency. Moreover, *M. charantia* extract caused bent back, scoliosis and edema in the yolk sac, bent tail tip embryos. These results strongly indicate the medicinal importance of *M. charantia*, which can lead towards the discovery of toxic substance or compounds with very promising bioactivities.

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