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Development and validation of a teratogenicity of golden oyster mushroom (*Pleurotus citrinopileatus* Singer.) extract on Zebrafish (*Danio rerio*)

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Abstract

Mushrooms are regarded as a rich source of nutritive components; yet, despite their high potential for bioactive chemicals, mushrooms can have harmful effects on developing organisms such as cytotoxicity and teratogenicity. This article examined the development and teratogenic effects of this mushroom on zebrafish (*Danio rerio*) as an animal model in order to determine its biosafety and potential as a source of chemicals with bioactivities. In order to get functional components for teratogenic and toxic effects in zebrafish embryos, hot water extraction was performed. Extract dramatically decreased the hatchability of zebrafish eggs at 1% or higher concentrations of water extract, as well as the heartbeat rate at 10% or higher concentrations. Embryos treated to a 5% concentration of the extract had growth retardation, which coagulated following treatment. Lethality was detected in the form of a coagulated embryo and slowed development. The biological activity of *P. citrinopileatus* provides information on its possible use as a bioactive chemical source. As a result, more research into other features is deemed important in future studies.

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Introduction

Philippines, due to its geographic location is consider as a ground rich of biodiversity, including fungal biodiversity. Hence, with diverse species, very limited knowledge and study are being conducted. The popularity of mushrooms has grown year after year due to their nutritional value and medicinal properties, which play an important role in human health. Mushrooms are thought to be an excellent source of a wide range of secondary metabolites with considerable bioactive properties (Mugdha *et al*., 2010).

P. citrinopileatus, often known as the golden oyster mushroom, is a popular cultivated fungus that can be produced in a variety of substrates. Because of its excellent nutritional content, ease of growing, and therapeutic benefits, this edible fungus is becoming increasingly popular (Musieba *et al*., 2011). Anti-inflammatory, immunostimulatory, anti-aging, antibacterial, anti-cancer, anti-fatigue, anti-inflammatory, antiviral, immunomodulatory (Chen *et al*., 2011), anti-tumor characteristics (Wang *et al*., 2005), and hence may have teratogenic and toxic consequences. Teratogens are agents that create anomalies in an organism's growth and development (Bertollini *et al*., 1993).

Therefore this study investigated the toxic and teratogenic effects of *P. citrinopileatus* water extracts on zebrafish eggs. The percentage mortality, hatchability, delayed development, and abnormalities of the exposed embryos were assessed, and the various morphological endpoints of the exposed embryos were reported.

Materials and methods

Mushroom Sample

P. citrinopileatus fruiting bodies were obtained from the Center for Tropical Mushroom Research and Development (CTMRD). Prior to extracting its functional components, the sample was dried and processed in a food processor.

Extraction of functional components

The functional components of *P. citrinopileatus* were recovered by hot water extraction using the approach of (Eguchi *et al*., 1999). The milled mushroom samples (40g) were extracted in 800 ml hot water in a water bath for 2 hours at 80 - 90°C. Filtration using filter paper No. 2 separated the solutions from the extract, which was then filter sterilized with 0.45 filters. By diluting the extract to embryo water medium, the extract filtrates were utilized to create the various treatment doses for the teratogenicity experiment (Thomas, 2000)

Spawning of Danio rerio

The procedures on spawning and fertilization were followed after (Nagel, 2002). A non-treated supply of tap water in an aquarium was utilized for zebra fish spawning with oxygen saturation, with adult females and males provided in a 1:2 ratio (1 female to 2 male). The temperature was 26 °C±1°C with a 12hour day/night light system. The zebrafish were fed dry flakes twice a day. For 12 hours, the aquarium was covered with a black plastic sheet and the fish were contained in a plastic mesh. A. To avoid egg release from cannibalism, the adult zebrafish were enclosed in a plastic mesh. After incubation in the dark, the eggs were exposed to light for another 12 hours.

Embryo-toxicity and teratogenicity assay

The toxicity and teratogenicity methodology utilizing zebrafish embryos was adapted from (Eguchi *et al*., 1999). Each well of the 12-well ELISA plate received three ml of each treatment concentration of *P. citrinopileatus* extract made with embryo water as a diluent (20%, 10%, 5%, 1%, and 0.5%) and control (embryo water). Four embryos at the segmentation stage were put into each well containing the various treatments. The plates were incubated at a temperature of 26°C ± 1°C. Teratogenic activity was assessed using a compound microscope at 40X magnification after 24 and 48 hours of incubation. The metrics proposed by were used to evaluate the morphology of zebra fish (Nagel, 2002).

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) in one way classification analysis. Means were compared using Least Significant Difference (LSD) at 5% level of significance. The Sirichai Statistics 6.07 program was used for analysis.

Results

Teratogenic effect of P. citrinopileatus

P. citrinopileatus extract made with embryo water as a diluent (20%, 10%, 5%, 1%, and 0.5%) and control (embryo water) were inserted in each well of the 12-well ELISA plate. At 24, and 48 hours after treatment, the embryonic development of zebrafish embryos cultured on a multiwell ELISA plate with varied doses of *P. citrinopileatus* hot water extract was observed. Hatching was performed 48 hours after embryo water treatment application (hpta) (control). The hatchability of embryos treated with 0.5 percent, 1 percent, or greater was considerably lower than that of the control (Table. 1).

Table 1. Zebrafish egg hatchability after 48 hours of treatment application.

Concentration (%)	Hatchability (%)
20	0.00^{c}
10	0.00^{c}
5	0.00^{c}
1	15.67^{b}
0.5	20.26^{b}
Control	100^{a}

*Means having the same letter are not significantly different

The varied extract concentrations had a substantial effect on heartbeat rate (Table. 2). Embryos cultured in embryo water had the most heartbeats per minute, with a mean of 162.78 per minute. Concentrations of extract 0.5 percent (114.26 heartbeat/min) and 1 percent (83.87 heartbeat/min) vary substantially from controls, with a declining trend as concentration increases. Similarly, to the hatchability percentage,

Table 2. Heartbeat rate of embryos during the pharyngual stage at various extract concentrations. Following 48 hours of therapy application.

Concentration (%)	Heartbeat (min)
20	0.00^{d}
10	0.00^{d}
5	0.00^{d}
1	83.87^{c}
0.5	114.26^{b}
Control	162.78^{a}

*Means having the same letter are not significantly different.

The presence of coagulation may be used to measure zebra fish death. As indicated in Table 3, there was no observed mortality in the lower concentrations of 0.5 percent and 1 percent observation period 24 hour after treatment application. Mortality was 100% after 48 hours of exposure at high concentrations of 5% and 10%, however although no mortality was found in low concentrations of 0.5 and 1% of extract at 24 hour post treatment application (hpta), there was an increase in mortality rate at 48 hour post treatment application (hpta).

Table 3. Mortality of zebrafish embryos after 24 and 48 hours of treatment application.

Concentration (%)	Observation Period (%)	
	24	48
20	100^{a}	100^{a}
10	100^{a}	100^{a}
5	100^{a}	100^{a}
1	0^{b}	37.27^{b}
0.5	0^{b}	28.41^{b}
Control	0^{b}	0^{c}

*Means having the same letter are not significantly different.

These findings imply that embryo mortality was determined by the amount of extract used and the period of exposure. Exposure of embryos to extract for a longer period of time and at higher concentrations results in increased mortality of zebrafish embryos.

P. citrinopileatus extract was shown to be fatal in zebrafish embryos, with death defined as coagulation and no visible heartbeat. embryos. The death rates of embryos after Fig. 1 depicts 48 hours of exposure in various extract concentrations. 1. It can be shown that the deadly effects of *P. citrinopileatus* extracts were dependent on depending on the amount and duration of exposure. The measures proposed by were used to evaluate morphological defects or deformities in zebra fish (Nagel, 2002). All embryos exposed to 0.5 percent and some embryos exposed to 1 percent of both extracts had various abnormalities (Fig. 1).

Malformations were found. Malformations observed at 1 percent or higher concentrations of extract included malformation of the eye, malformation of the tail, craniofacial malformation (5 percent), growth retardation, and more serious developmental inhibition of embryos (no head and tail formed) when incubated at higher concentrations of extract (5 percent).

Tail abnormalities were discovered in a 24-hpta embryo. Teratogen exposure resulted in delayed growth and developmental defects in zebrafish embryos.

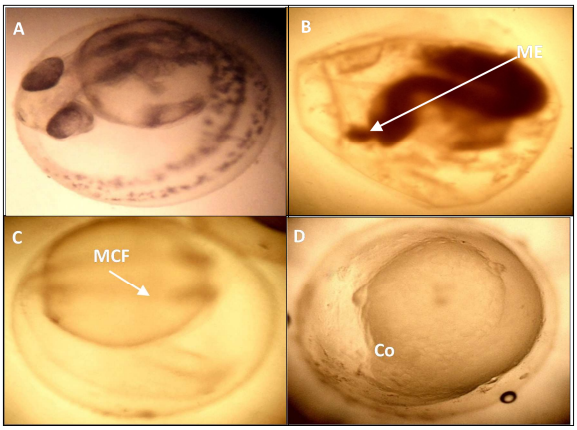

Fig. 1. Lethal and sublethal effects of *P. citrinopileatus* extract on zebrafish embryos (A) Control concentration after 48 hours (hours post treatment application). (B) Embryo with ME-malformation of the eye at 1% concentration at 24 hpta. (C) After 24 hpta, embryo with MCF-malformation of cranial development at 5% concentration. (D) CO-coagulation formation in a 48-hpta embryo at 10%.

Discussion

Failure to hatch was the most significant and important phase in the development of zebrafish embryos (Herrera, Dee, Ipulan. 2010a). Teratogenic influences might have an impact on embryo hatchability (Herrera, Dee, & Ipulan, 2010b). Thus, the inability of embryos to hatch at high concentrations of 0.5 percent or more shows that the embryos are already dead or that the teratogenic impact in the embryos inhibits their capacity to hatch.

Heartbeat regularity is a criterion for heart function that is linked to cardiotoxicity in humans (Chan *et al.*, 2009). Heartbeat regularity is also linked to cardiotoxicity (Chan *et al.*, 2009) and may result in abrupt cardiac death. The heartbeat rate was also shown to be concentration dependent. The extract drastically reduces the heartbeat of zebrafish embryos, perhaps leading to embryo mortality.

Coagulation was used to estimate embryo mortality when no apparent organ structures were identified (Herrera, 2007), and this provided the foundation for detecting coagulated eggs in our work.

Conclusion

The current study focuses on the toxic and teratogenic effects of *P. citrinopileatus*. Teratogenic and embryotoxic activity in zebrafish is related to dose and period of exposure. The degree and severity of the deformities, as well as the deadly toxic consequences, are concentration dependent and time dependent.

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