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Performance of mycelial biomass and exopolysaccharide from Malaysian *Ganoderma lucidum* for the fungivore red hybrid Tilapia (*Oreochromis* sp.) in Zebrafish embryo

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ABSTRACT

Natural mycelial biomass (MB) and extracted exopolysaccharide (EPS) from the pre-grown Malaysian *Ganoderma lucidum* mushroom are both considered as high-end materials due to their high commercial value in the aquaculture industry. To evaluate their potential toxicity as a fish-feed supplement for the fungivore red hybrid Tilapia (*Oreochromis* sp.), both MB (250 – 5000 μg/mL) and EPS (62.5 – 3000 μg/mL) were subjected to zebrafish embryo toxicity (ZFET) assay, and the effects on zebrafish embryos (ZE) early development were analyzed between 24 – 120 hours of post-exposure (HPE). MB and EPS showed no toxic effect towards the ZE with LC_{50} of 1650 μg/mL and 2648.38 μg/mL, respectively. MB at concentrations between 250 – 5000 μg/mL and EPS at 3000 μg/mL showed no significant changes in ZE hatching. No significant changes in the ZE heart rate were detected following treatment with both tested compounds (MB: 250 – 2000 μg/mL and EPS: 62.5 – 3000 μg/mL) as compared to untreated embryos (135.5 beats/min). Furthermore, teratogenic effects of both MB and EPS (< 3000 μg/mL) on zebrafish embryonic development were not observed. Together, both natural compounds MB and EPS can be considered non-toxic, suggesting that these can be safely applied as feed substances in the fish-feed aquaculture industry.

1. Introduction

Aquaculture is the fastest-growing food-producing industry globally (Fečkaninová et al., 2017; O'Neill et al., 2019a, 2020). It affords one of the most sustainable forms of edible protein with a low carbon footprint (Liu et al., 2017; Ruis-Salmon et al., 2020). Aquaculture rapid expansion has resulted in response to a dramatic increase in global population and commensurate demand for food, which highlights trajectory towards intensive sustainable products and resource efficiency (Freitas et al., 2019). In 2014, aquaculture production reached 73.8 M tonnes (Huynh et al., 2017), and now accounts for ~50 % of fishery products produced for human consumption (Liu et al., 2017). Aquaculture expansion has been driven by enhanced process efficiencies that includes addressing operational performance water quality, disease mitigation, nutrition and health of farmed fish including trend towards achieving natural or organic status (O'Neill et al., 2020; Tahar et al., 2018a, b). Ample evidence supports the application of the medicinal mushroom *Ganoderma lucidum* in various areas including wastewater treatment (Hanafiah et al., 2019), natural drug discovery and therapeutics (Smith et al., 2002; Sullivan et al., 2006; Wan-Mohtar et al., 2017), food-biomass chain (Stamets, 2011), protein-rich food (Rahmann et al., 2019) that includes future intensive use in aquaculture. These potential applications are largely attributed to its high protein biomass (Wan-Mohtar et al., 2018) and extracted exopolysaccharide (EPS) (Hassan et al., 2019) contents. However, *G. lucidum* application is still scarce in the aquaculture industry, and the closest counterparts are by utilising

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the fruiting bodies of Shiitake for Rainbow trout feed (Baba et al., 2015), fingerlings of Carps (Paripuranam et al., 2011), and chicken feed (Giannenas et al., 2010). So far, aquaculture application in the fish-feed industry using mushroom biomass-EPS remains scarce and warrants further discovery.

Many scientists considered *G. lucidum* biomass-EPS as a high-end material with high potential as an aquaculture feed (Rahmann et al., 2019). However, there is a need to evaluate the toxicity of biomass-EPS extract prior to their development and usage as an alternative feed or food supplement before commercialisation. This constitutes the first study to report on the use of zebrafish embryo toxicity (ZFET) assay as primary safety evaluation tool before pre-clinical testing according to national and international standards (Sewell et al., 2017). This assay is applicable as the early stages of embryonic development are usually more sensitive to toxicological effects. In this study, ZFET assay was performed using seven different concentrations of MB and EPS to obtain its LC_{50}. Thereafter, their effects on hatching, heart rate and development of Zebrafish embryos were evaluated.

2. Material and methods

2.1. G. lucidum sample preparations

The fruiting body of the medicinal mushroom was packaged and stored in an optimised packaging condition to retain its freshness (Wan-Mohtar et al., 2019). Stock solutions [5 mg/mL of mycelial biomass (MB): 3 mg/mL of exopolysaccharide (EPS)] were cultivated and extracted from *G. lucidum* QRS 5120 (Supramani et al., 2019a, b) using the original method reported previously (Wan-Mohtar et al., 2017). Working solutions were prepared by diluting the stock mycelial biomass extract in embryo media (Danio-SprintM solution) in 2-fold serial dilutions to obtain seven concentrations ranging from 250 − 5000 μg/mL (MB) and 62.5 − 3000 μg/mL (EPS) in a 96-well microplate. Embryos cultured in embryo media only (Danio-SprintM solution) was used as the control (untreated).

2.2. Zebrafish maintenance and breeding

For the zebrafish model, the breeding and maintenance of zebrafish (Georga and Koumoundouros, 2010) broodstocks were performed with the permission of the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia, Selangor, Malaysia. Briefly, eggs were collected following breeding of a pair of adult zebrafish. The eggs were washed and incubated in the embryo media, Danio-SprintM solution for approximately 2 h. Dead or coagulated embryos were discarded, and healthy fertilised embryos were selected for the assay.

2.3. Zebrafish embryo toxicity (ZFET) assay

The zebrafish embryo toxicity assay was carried out according to the Organization for Economic Cooperation and Development (OECD) guideline for fish embryo toxicity (FET) test (OECD, 2013). Briefly, zebrafish embryos (one embryo/well) at 24 h post-fertilization (24 hpf) were exposed to each concentration of MB and EPS extracts (200 μL) ranging from 250 − 5000 μg/mL in a 96-well microplate. The control (untreated) and each treatment group of MB and EPS were performed in 12 replicates. Following treatment or exposure to the extracts, the embryos were incubated at room temperature (25 − 28 °C) for five days. The cumulative mortality and developmental malformations of embryos and larvae were observed and determined every 24 h between 0 − 120 hours post-exposure (hpe). The survival rate, hatching rate, heart rate, morphological malformation or teratogenic defects were observed, and morphological changes were captured using an inverted microscope attached to a digital camera (THUNDER Imager 3D Live Cell & 3D Cell Culture & 3D Assay, Leica Microsystems GmbH, Wetzlar, Germany). The heartbeat was counted from three selected embryos using a stopwatch for 1 min. Lethal endpoints were characterized by coagulation and no heartbeat. Developmental anomalies include pericardial oedema, yolk sac oedema, non-hatched, curved body and bent tail. Based on a previous study (Ohikhena et al., 2016) which used a brine shrimp lethality test as a reference, the extracts would be considered non-toxic if its LC_{50} value is greater than 1000 μg/mL. If the LC_{50} value lies between 500 − 1000 μg/mL, the extract would be considered to have weak toxic effects, whereas values above 500 μg/mL are considered toxic.

2.4. Calculation

All graphs were generated using GraphPad Prism version 7.0 (GraphPad Software, Inc.). The lethal concentration at 50 % (LC_{50}) of treated samples toward zebrafish embryos was also measured using the same software. Heart rate was presented as mean ± standard error of the mean (S.E.M) from three different embryos. The data was statistically analyzed by one-way analysis of variance (ANOVA) with a post hoc test using Dunnett's Multiple Comparison. The changes between the means of the treated group were considered statistically significant if $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared to zebrafish embryos in embryo media only (untreated).

3. Results

3.1. The effects of MB and EPS on the survival rate of zebrafish embryos

The effects of each MB and EPS extract on zebrafish embryo survival rate were analyzed between 0–120 hpe. The survival rate of embryos (before hatch) and larvae (after hatch) treated with MB extract was determined for five days. Fig. 1a shows that untreated embryos had a 100 % survival rate between 0–120 hpe. The survival rate dropped slightly (90 %) when exposed to MB extract at concentrations < 1000 μg/mL, while at concentrations > 2000 μg/mL, a low survival rate (< 30 %) was observed at 72 hpe. No embryo survived after 72 hpe at concentrations > 4000 μg/mL. Fig. 1b shows the survival rate of embryos (before hatch) and larvae (after hatch) treated with EPS (62.5 − 3000 μg/mL) over five days. Untreated embryos (control) had a 100 % survival rate between 0–120 hours of hpe. The survival rate dropped slightly (90 %) when exposed to EPS extract at concentrations < 2000 μg/mL, while at 3000 μg/mL, a low survival rate (< 40 %) was observed at 48 hpe. No embryo survived after 72 hpe.

3.2. The effects of MB and EPS on the mortality rate of zebrafish embryos

Overall, the lethal effects of MB and EPS extracts were dose- and time-dependent. In Fig. 2, EPS and MB at concentrations below 2000 μg/mL and 1000 μg/mL, respectively, showed a high survival rate (90 %) of zebrafish embryos. However, 3000 μg/mL EPS and 2000 μg/mL MB yielded a low survival rate, and none survived after 72 hpe. Hence, the lethal concentration at 50 % (LC_{50} value) of zebrafish embryos exposed to MB was 1650 μg/mL, while for the EPS extract, the LC_{50} value is 2648.38 μg/mL.

3.3. The effects of MB and EPS on the hatching rate of zebrafish embryos

Accordingly, varying concentrations of extract would affect the hatchability of the embryo. The percentage of hatchability decreased with increasing concentrations of extracts. Fig. 3 shows the hatching rate of zebrafish embryo (Fig. 3a) upon MB (250 − 5000 μg/mL) and (Fig. 3b) EPS (62.5 − 3000 μg/mL) treatments at 0–120 hpe. No significant changes were observed in the hatching rate upon treatment with MB extract at concentrations < 1000 μg/mL. However, at 4000 μg/mL, the hatching rate was reduced to < 60 %. Further reduction was observed (10 % hatching rate) when treated with MB extract at the concentration of 5000 μg/mL, reflecting a high mortality

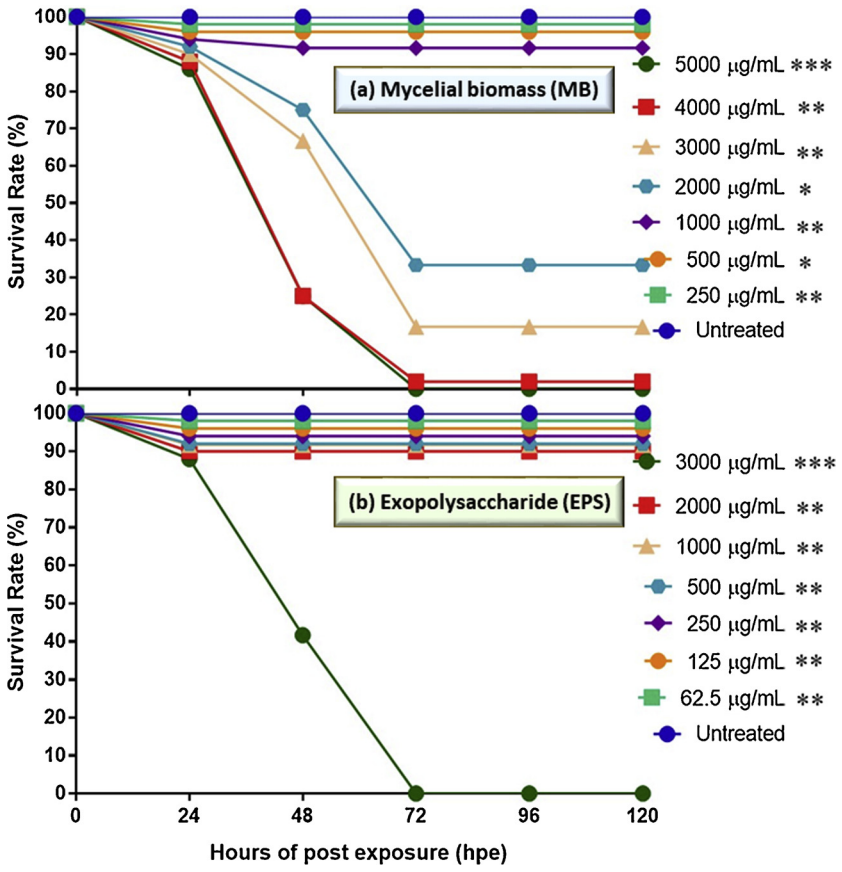

Fig. 1. The survival rate of zebrafish embryos at 0 to 120 h after exposure to the *Ganoderma lucidum* strain QRS 5120; (a) mycelial biomass (MB) extract at concentrations of 250-5000 µg/mL and to (b) exopolysaccharide (EPS) at concentrations of 62.5-3000 µg/mL. The sample was compared to the untreated group (zebrafish embryos in media only). The number of embryos tested for each concentration was 12 animals. The changes between the means of the treated group were considered statistically significant if *P < 0.05, **P < 0.01 and ***P < 0.001 as compared to zebrafish embryos in embryo media only (untreated).

rate of zebrafish embryos (24 hpe). On the other hand, less than 80 % of the embryos hatched on the second day of treatment (48 hpe) with EPS at concentrations > 1000 µg/mL. However, zebrafish larvae treated with EPS at concentrations 3000 µg/mL showed the lowest hatching rate (< 30 %) due to the high mortality rate after 72 hpe.

3.4. The effects of MB and EPS on the heart rate of zebrafish embryos

The heart is the primary functional organ during the development of many model organisms, including zebrafish (Bakkers, 2011). Based on Fig. 4, the heart rate of zebrafish larvae at 96 hpe (4 days) for both MB (Fig. 4a) and EPS (Fig. 4b) treatments were recorded at 135 beats/min. This data was in accordance with a previous report in which the normal heart rate of zebrafish embryo is much closer to that of humans at 120–180 beats per minute (Baker et al., 1997). Both extracts at lower concentrations (compared to higher concentrations in Fig. 3) ranging between 250 – 2000 µg/mL for MB, and 62.5 – 2000 µg/mL for EPS, showed no significant difference towards the heart rate of zebrafish larvae at 96 hpe. Since MB extract at 3000, 4000 and 5000 µg/mL showed very little to no survival at 96 hpe, the heart rate of zebrafish

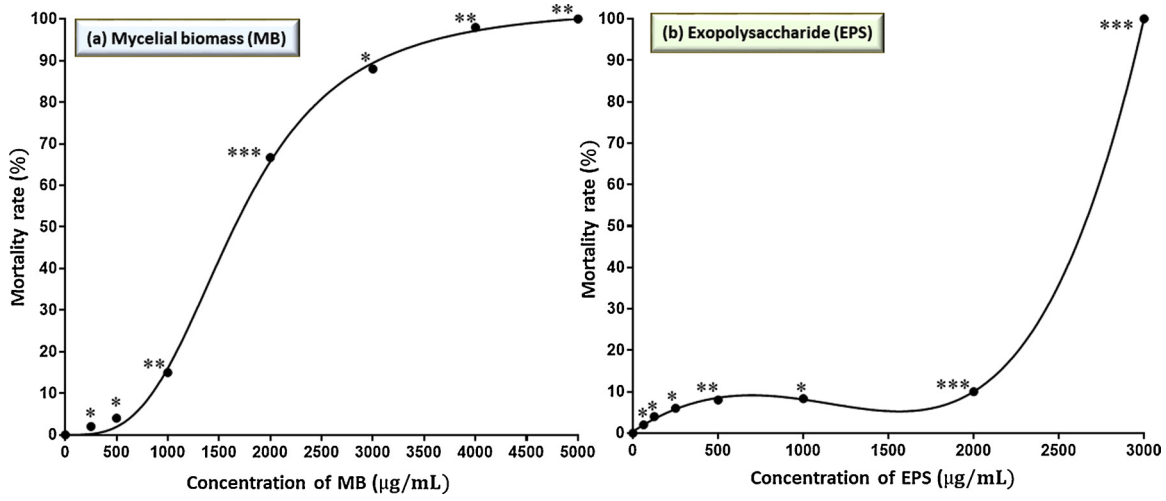

Fig. 2. Effect of *Ganoderma lucidum* strain QRS 5120 (a) mycelial biomass (MB) extract at concentrations of 250-5000 µg/mL and (b) EPS at concentrations of 62.5-3000 µg/mL on zebrafish embryos mortality rate after 120 h of post-exposure (hpe). The LC_{50} value of extract towards zebrafish embryos was 1650 µg/mL (MB) and 2648.38 µg/mL (EPS), respectively. The number of embryos tested for each concentration was 12 animals. The changes between the means of the treated group were considered statistically significant if *P < 0.05, **P < 0.01 and ***P < 0.001 as compared to zebrafish embryos in embryo media only (untreated).

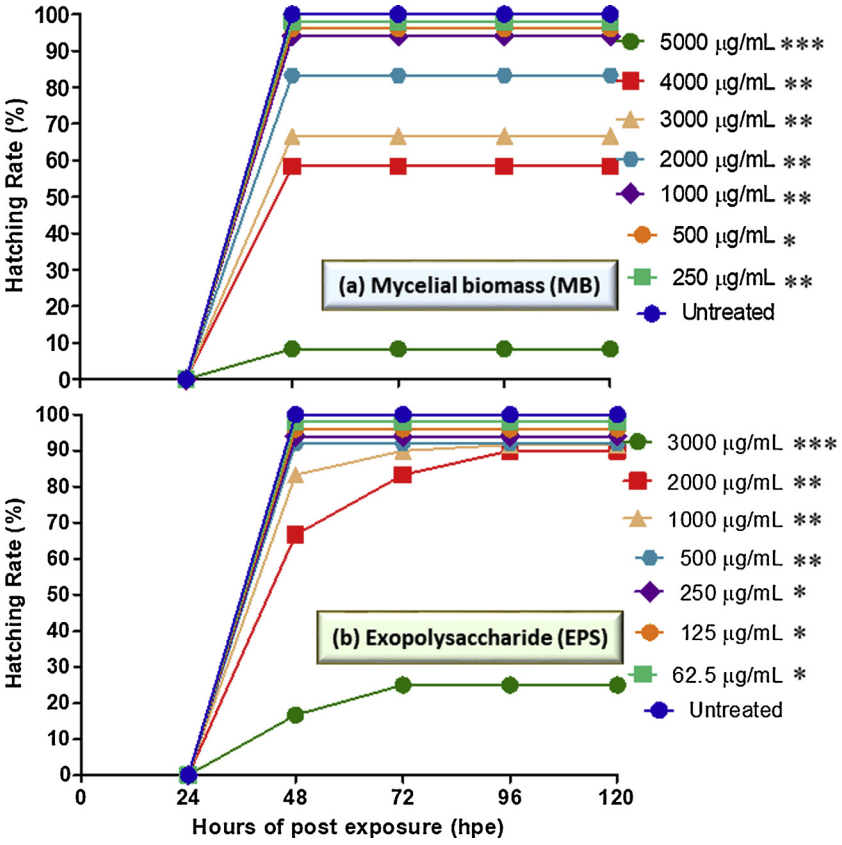

Fig. 3. Hatching rate of zebrafish embryos at 0 to 120 h of exposure with the extracts of *Ganoderma lucidum* strain QRS 5120 (a) MB (250-5000 µg/mL) and EPS (62.5-3000 µg/mL). The sample was compared to the untreated group (zebrafish embryos in media only). The number of embryos tested for each concentration was 12 animals. The changes between the means of the treated group were considered statistically significant if *P < 0.05, **P < 0.01 and ***P < 0.001 as compared to zebrafish embryos in embryo media only (untreated).

larvae at these concentrations were not determined. Similarly, the EPS at 3000 µg/mL also showed a high mortality rate (100 %) at 96 hpe; therefore the heart rate of zebrafish larvae was not determined.

3.5. The effects of MB and EPS on the morphology of zebrafish embryos and larvae development

The possible morphological defects of embryos and larvae were observed and measured from 0 hpe to 120 hpe. Fig. 5 shows no visible teratogenic effect on the embryos and larvae at 120 h after exposure to MB at concentrations < 3000 µg/mL. Fig. 6 shows that EPS (62.5 – 3000 µg/mL) also did not have teratogenic effects on the development of zebrafish embryos before and after hatch. These results suggest that both MB and EPS have no teratogenic effects on the development of zebrafish embryos before and after hatching.

In Fig. 7 and Fig. 8, zebrafish embryo and larvae development were unaffected when treated with MB extract at concentrations of 1000 µg/mL and 2000 µg/mL EPS from 0 hpe to 120 hpe. However, various abnormalities were observed as the concentration increased to 5000 µg/mL MB and 3000 µg/mL EPS (Fig. 9 and Fig. 10). One of the most distinct abnormalities observed includes tail malformation, which was observed in embryo treated with EPS and MB at 72 hpe. Furthermore, coagulated embryos were also observed with both treatments, resulting in unhatched embryos after 120 hpe.

4. Discussion

In this experiment, both MB and EPS extracted from liquid-fermented *Ganoderma lucidum* were tested for acute toxic effects on zebrafish embryos. MB is derived from edible mushroom species and is a popular high-end product as a dietary supplement (Wasser et al., 2000). MB also could be used in the food industry such as for flavours (Hadar

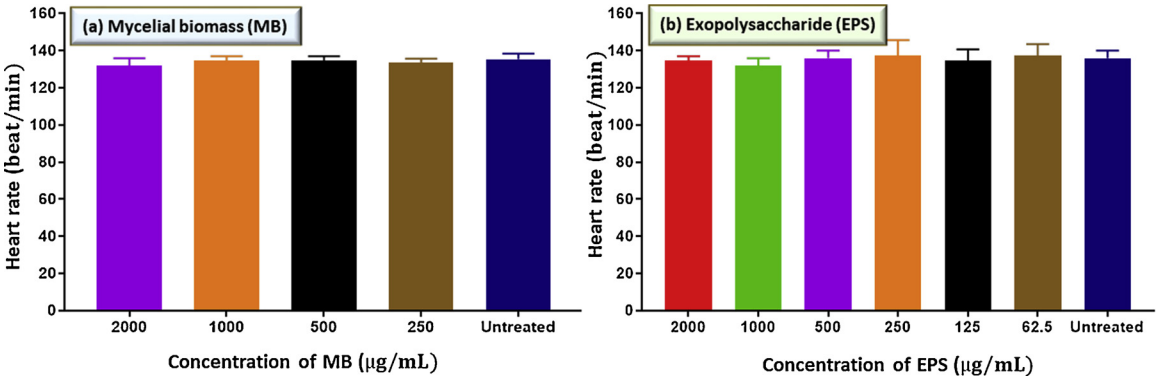

Fig. 4. Effect of *Ganoderma lucidum* strain QRS 5120 (a) MB extract (250-2000 µg/mL) and (b) EPS extract (62.5-2000 µg/mL) on the heart rate of zebrafish embryos at 96 h of post-exposure. *$P < 0.05$ significantly different from the untreated group (zebrafish embryos in media only). The number of embryos tested for each concentration was three animals.

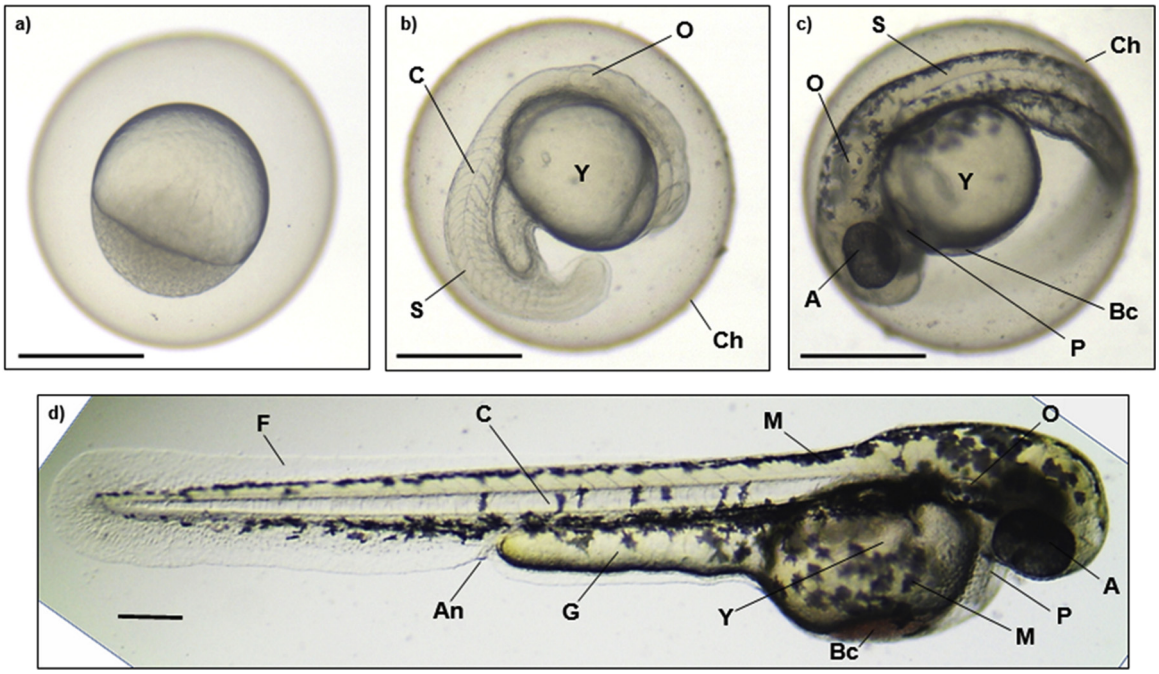

Fig. 5. Images of normal zebrafish embryogenesis showing stages of development at different hours of post-fertilization (hpf) captured using inverted microscope treated with MB extracts (< 3000 µg/mL) of *Ganoderma lucidum* strain QRS 5120. a) Blastula period (4 hpf); b) Segmentation period (24 hpf); c) Pharyngula period (48 hpf); d) Hatching period (72 hpf). Scale bar = 0.5 mm. A – eye anlage; An – anus; Bc – blood cells; C – chorda; Ch – chorion; F – fin; G – gut; M – melanophores; O – ear bud; P – pericard; S – somites; Y – yolk sac.

and Dosoretz, 1991) and other metabolites such as enzymes and EPS (Lin and Yang, 2019).

In this toxicity test, zebrafish embryos or larvae were used as an animal model. This model offers several advantages (Caballero and Candiracci, 2018). First, zebrafish embryos are demersal, which they settle to the bottom of the 96-well plate and make direct contact with the mycelial biomass, mimicking the direct contact between zebrafish embryo and the mycelial biomass. Second, transparency and extra-uterine development can be examined, allowing direct observation of phenotypic changes during embryonic development. Third, zebrafish share many cellular and physiological characteristics with higher vertebrates. Thus, toxicological results can be compared with those from studies on developmental toxicity in mammals. Assessing the embryotoxic and teratogenic effects of certain compounds on the development

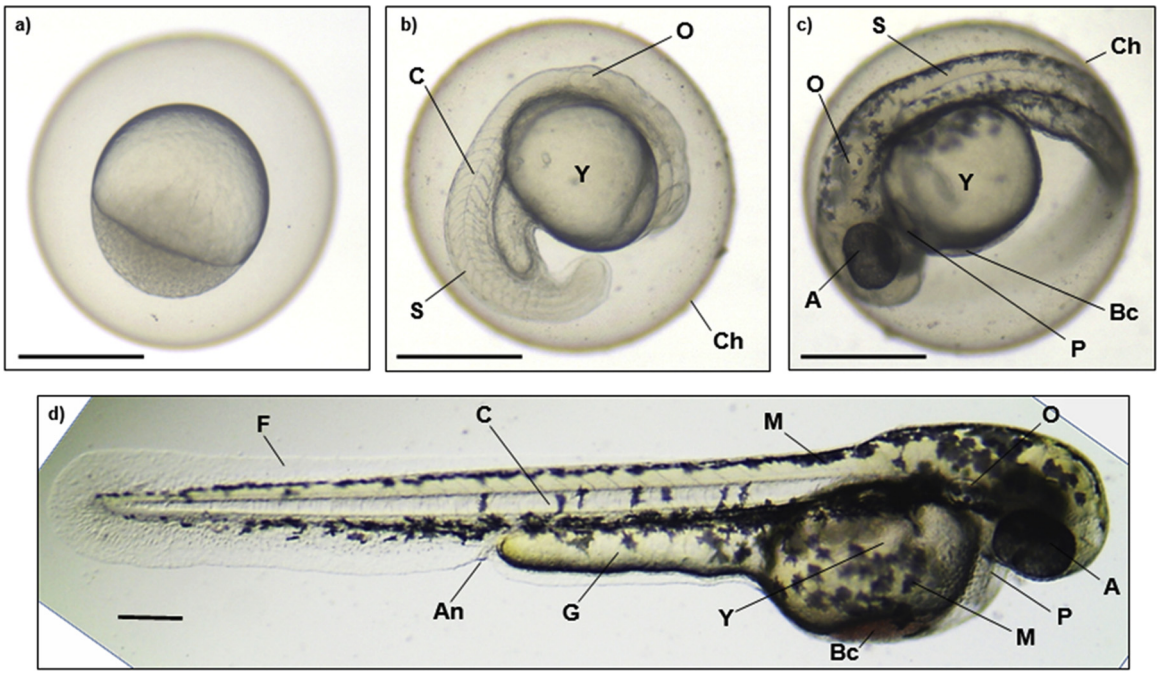

Fig. 6. Images of normal zebrafish embryogenesis showing stages of development at different hours of post-fertilization (hpf) captured using inverted microscope treated with EPS extracts (62.5-3000 µg/mL) of *Ganoderma lucidum* strain QRS 5120. a) Blastula period (4 hpf); b) Segmentation period (24 hpf); c) Pharyngula period (48 hpf); d) Hatching period (72 hpf). Scale bar = 0.5 mm. A – eye anlage; An – anus; Bc – blood cells; C – chorda; Ch – chorion; F – fin; G – gut; M – melanophores; O – ear bud; P – pericard; S – somites; Y – yolk sac.

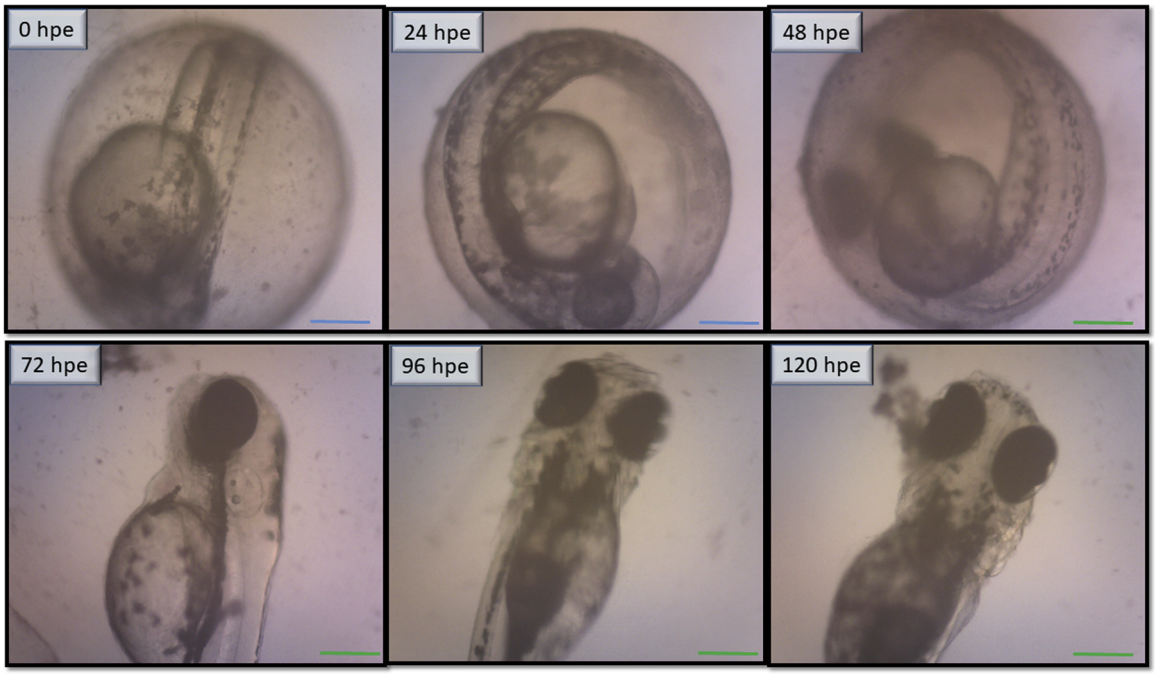

Fig. 7. Images of normal zebrafish embryo and larvae development exposed with *Ganoderma lucidum* strain QRS 5120 MB extracts at a concentration of 1000 μg/mL from 0 to 120 hpe of treatment. Images were captured using an inverted microscope at 100X (blue bar) and 40X magnification (green bar). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

of the embryo is essential to determine the amount that is safe for consumption. Several products derived from plants and fungi are gaining popularity in the global health market and claimed to have a pharmacological effect, although their toxicology profile is still dubious.

Based on our recent study, fertilised embryos were exposed to various concentrations of *G. lucidum* extract, EPS (62.5 − 3000 μg/mL) and MB (250 − 5000 μg/mL). Overall, EPS at concentrations < 2000 μg/mL and MB at < 1000 μg/mL did not cause delay hatching towards the zebrafish embryo and with the survival rate of 90 % at 24–120 hpe. Besides, there were no significant differences in both EPS and MB at concentration < 2000 μg/mL on the heart rate as compared to untreated embryos. Meanwhile, there were visibile teratogenic effects on zebrafish embryonic development at the concentrations of < 3000 μg/mL and (62.5–3000 μg/mL) in MB and EPS, respectively.

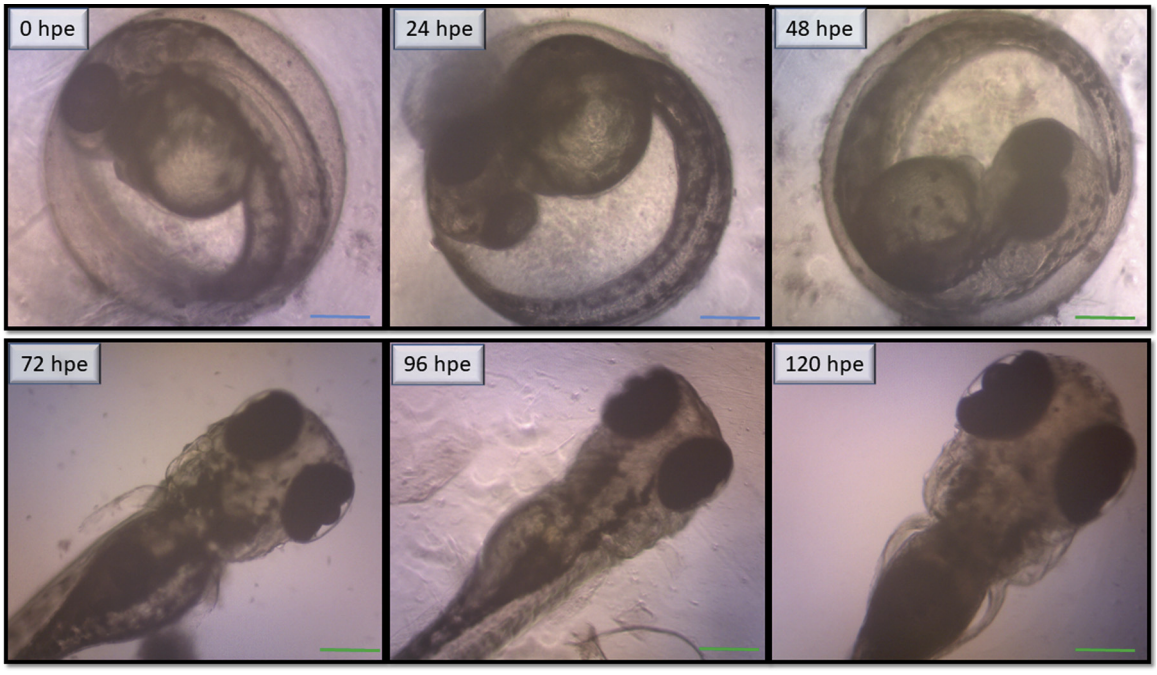

Fig. 8. Images of normal zebrafish embryo and larvae development exposed with *Ganoderma lucidum* strain QRS 5120 EPS extracts at a concentration of 2000 μg/mL from 0 to 120 hpe of treatment. Images were captured using an inverted microscope at 100X (blue bar) and 40X magnification (green bar). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

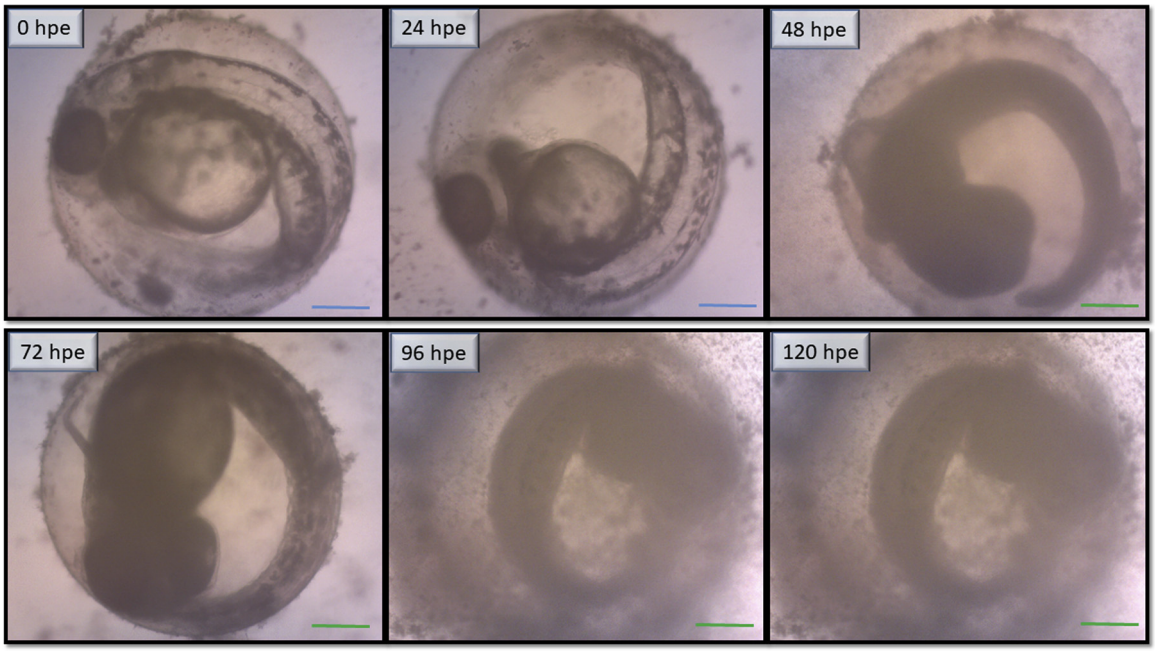

Fig. 9. Images of zebrafish morphology after exposed with *Ganoderma lucidum* strain QRS 5120 MB extracts at a concentration of 5000 μg/mL started from 0 to 120 hpe of treatment. Images were captured using an inverted microscope at 100X (blue bar) and 40X magnification (green bar). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

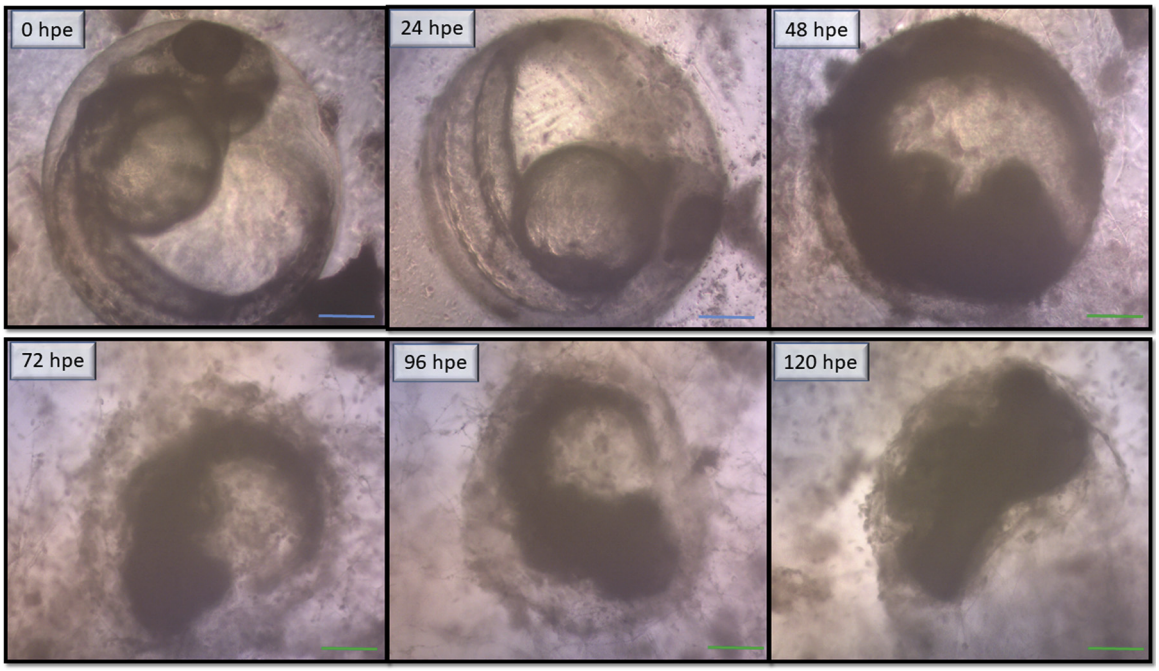

Fig. 10. Images of zebrafish morphology after exposed with *Ganoderma lucidum* strain QRS 5120 EPS extracts at a concentration of 3000 μg/mL started from 0 to 120 hpe of treatment. Images were captured using an inverted microscope at 100X (blue bar) and 40X magnification (green bar). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Based on the assay, EPS reflects a higher LC_{50} value (2648.38 μg/mL), which indicates better choice as compared to the MB (1650 μg/mL). Although both extracts (EPS and MB) are from *G. lucidum*, they may contain different amount or composition of compounds as they are originated from different parts (Huang et al., 2015; Ma et al., 2018; Zhao et al., 2010). Previous study suggested that exopolysaccharides isolated from the *G. lucidum*EPS exhibit a broad range of bioactivities, including anti-inflammatory, hypoglycemic, antitumorigenic, and immunostimulating effects (Ahmadifar et al., 2019), which are higher than the MB and fruiting bodies (Kozarski et al., 2019).

Numerous studies have shown that zebrafish embryo could be used as a way to explore the medicinal potential of plants and fungi extracts (Chen et al., 2017; Polednik et al., 2018; Vranic et al., 2019). One of the reasons for the toxicity of plant extract towards aquatic organisms, including zebrafish is they could disrupt the balance of water chemistry when the plant compounds dispersed in water (Muniandy, 2018). Another reason including the presence of flavonoids, which might induce cytotoxicity, although the low impact was observed in mammals (Bugel

et al., 2016). Therefore, the presence of flavonoid in the mycelia of *G. lucidum* could be the reason for the toxicity effect in the zebrafish embryo at 2000 − 3000 μg/mL of MB-EPS.

Morphological abnormalities, including tail malformation, could limit the embryo's ability to break the chorion and hatch out. Also, the absence of heartbeat and coagulation embryos can be seen as lethal effects. The previous study by Dulay et al. (2012) reported that tail malformation could be observed in zebrafish embryos exposed to the 1% extract (10 mg/ml) of *G. lucidum* fruiting body. Such response indicates that < 1% concentration is the safety limit for *G. lucidum* fruiting body extract, which is less toxic than the current study reported for MB and EPS. However, polysaccharides yield from fruiting bodies of *G. lucidum* is lower in amount and relatively costly compared to the exopolysaccharides, which limit the health-promoting and therapeutic benefit (Ye et al., 2018).

Aside from *G. lucidum*, several other mushrooms have been tested on the toxicity effect towards zebrafish embryo. Termite mound mushroom *Termitomyces clypeatus* exposed to the zebrafish embryos at the concentration of 0.1 % (De Castro et al., 2016) or higher resulted in significantly low hatchability (less than 50 % after 48 hpe with the presence of teratogenic effects (Wu et al., 2020). In other studies, zebrafish embryos treated with *Pleurotus ostreatus* ethanol extract at 2.5 % and 5% recorded full mortality after 12 h while tail malformation and delayed growth can be observed at 1% concentration De Castro and Dulay (2015).

It is indispensable to accept the ingredients that contain non-nutrient factors such as bioactive food compounds that have been associated with promoting health (Watts et al., 2016). Several studies have reported that *G. lucidum* polysaccharides can be used as feed supplements on aquatic species to improve growth and immunity. This includes giant freshwater prawn (*Macrobrachium rosenbergii*) (Mohan et al., 2019) and grass carp (*Ctenopharyngodon Idella*) (Chithra et al., 2016) with acceptable concentrations ranging from 1.0–1.5 g/kg, which enhance growth and innate immune response. Thus, in order to include the EPS and MB extracts from *G. lucidum* into aquaculture feed, toxicity reports are essential to ensure the safety concentration on the animals. Hence, the present results may provide useful data for assessing the potential health risks of the MB-EPS consortia. However, further tests need to be carried out to evaluate the LC_{50} value of MB-EPS extract on bigger animals (*e.g.*, rodent. rabbit, trout, carp, and adult tilapia before it can be developed as intended uses.

Recent *in vitro* and *in vivo* toxicity assessment of *G. lucidum* mycelial extracts are shown in Table 1. As reported, only two studies depicted the non-toxic verification of EPS from *G. lucidum* using normal human prostate cell line (Wan-Mohtar et al., 2016) and normal human lung cell (Chung et al., 2001) while one study gave verification on the non-toxic mycelial biomass powder using normal Wistar outbred white male rats (Vitak et al., 2015). Hence, the current study gave the only verification for both non-toxic mycelial biomass and EPS using the zebrafish model. Together, this study clarified the safe use of *G. lucidum* mycelial extracts *via* the Zebrafish model, which are small, robust, economical, fast, transparent, efficient early development study, similar genetic structure to humans and akin significant organs and tissues as humans.

MB and EPS extract from the mycelium of cultivated *Ganoderma lucidum* showed no toxicity effect towards the zebrafish embryos with LC_{50} value of 1650 μg/mL and 2648.38 μg/mL, respectively. Both MB and EPS extract did not cause delay hatching towards the zebrafish embryo and with a survival rate of 90 % at 48 hpe. Both compounds gave no significant difference in the heart rate at concentration < 2000 μg/mL as compared to untreated embryos. Besides, there were no teratogenicity effects on zebrafish embryonic development at concentration (62.5–3000 μg/mL) and (< 3000 μg/mL) in EPS and MB respectively. Thus, this warranted that both MB and EPS are non-toxic.

In conclusion, this constitutes the first study to report on the use of zebrafish embryo toxicity (ZFET) assay as a primary safety evaluation tool for demonstrating toxicology efficacy of natural mycelial biomass and extracted exopolysaccharide from the pre-grown Malaysian *G. lucidum* mushroom for aquaculture feed usage. Findings supported no toxicity in a suite of toxicity tests, thus supporting usage as a potential fish-feed supplement for the fungivore red hybrid Tilapia (*Oreochromis* sp.). Future studies should be extended to consider use in commercial deployment and extending to other high-value fish farmed in aquaculture.

CRediT authorship contribution statement

Norhidayah Mohd Taufek: Methodology, Validation, Writing - original draft, Writing - review & editing. **Hanis H. Harith:** Data curation, Formal analysis, Investigation, Writing - review & editing. **Muhamad Hafiz Abd Rahim:** Validation, Writing - original draft, Writing - review & editing. **Zul Ilham:** Data curation, Formal analysis, Investigation, Writing - review & editing. **Neil Rowan:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing - original draft, Writing - review & editing. **Wan Abd Al Qadr Imad Wan-Mohtar:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the

Table 1
Comparison with published non-toxicity assessment of mycelial biomass and exopolysaccharide from the mushroom *Ganoderma lucidum*.

Source	Non-toxicity test	Non-toxic concentrations		References
		Mycelial biomass (MB)	Exopolysaccharide (EPS)	
G. lucidum QRS 5120	*In vivo* – Zebrafish embryos and larvae	1650 μg/mL	2648.38 μg/mL	*Current study*
G. lucidum BCCM 31549	*In vitro* – normal human prostate cell line (PN2TA)	NA	500 μg/mL	(Wan-Mohtar et al., 2016)
G. lucidum	*In vitro* – normal human lung cell (WRL68)	NA	1000 μg/mL	(Chung et al., 2001)
G. lucidum Mycolivia-3 CBC 13744	*In vivo* – Wistar outbred white male rats (normal)	1 g/kg / mL	NA	(Vitak et al., 2015)

*NA = not available.

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