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Development and transportation pathway of Alpinia galanga oil loaded Self-Micro Emulsifying Drug Delivery Systems (SMEDDS) for fish anesthesia

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Alpinia galangal, an important edible plant in family Zingiberaceae is commonly used in Asian folk medicinal remedies. The essential oil of A. *Galanga* Rhizomes (AGO) has many biological activities e.g., antioxidant, antibacterial, antifungal and anti-inflammatory actions. However, the poor water miscibility of AGO causes the limitation of its clinical use in both human and animals. The alcohol used to dissolve AGO for fish anesthesia always causes hyperactivity in fish. The aim of this study was to solve this problem by developing Self-Micro Emulsifying Drug Delivery Systems (SMEDDS) of AGO. Pseudoternary phase diagrams of AGO were constructed to identify the best AGO-SMEDDS formulation. It was found that the AGO-SMEDDS composed of 20.0% AGO and 53.3% tween 80 and 26.7% ethanol had a mean droplet size of 82 nm after dispersing in distilled water. The anesthetic activity of the developed AGO-SMEDDS in koi (*Cyprinus carpio*) was evaluated in comparison with an AGO ethanol solution on the induction time required to reach the surgical anesthetic stage in which the fish stop all swimming activity and show loss of equilibrium and responsiveness. Results showed that the induction times of the fish receiving 200, 300 and 400 mg/L AGO-SMEDDS were 233, 130 and 112 seconds, respectively. Importantly, AGO-SMEDDS showed significantly higher anesthetic activity than the AGO ethanol solution which showed the induction times of 303, 207 and 167 seconds, with the same dose of AGO, respectively. The transportation pathway of AGO was investigated using a fluorescence microscope. AGO was labeled with Nile red. The brain, gills and skin of fish showed red fluorescent spots without auto fluorescence phenomena compared to unlabeled AGO. This result suggests that the AGO entered the fish via gills and skin and was transported to the brain where the anesthetic effect took place.

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