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Functional significance of olive flounder (*Paralicthys olivaceus*) plasma derived exosomes in wound healing activity

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Background: Exosomes are a group of extracellular vesicles containing DNA, RNA, proteins, and lipids, and functioning for intercellular communication and homeostasis. Exosomes have been highly incorporated for the therapeutic and diagnosis applications in scientific field due to their characteristic features.

Objective: In this study, we isolated exosomes from olive flounder (*Paralichthys olivaceus*) plasma and observed its wound healing and tissue regeneration activity.

Methods & Materials: Exosomes were isolated by two isolation techniques and were characterized using nano tracking particle analysis, Transmission Electron Microscopy (TEM), and immunoblotting. *In vitro* cytotoxicity was evaluated on Fathead Minnow (FHM) fish epithelial and murine macrophage Raw 264.7 cells and *in vivo* toxicity was determined on zebrafish larvae. Gene expression patterns of FHM cells treated with exosome were tested by qRT-PCR. Wound healing activity was determined by *in vitro* scratch wound healing assay and *in vivo* zebrafish larvae fin regeneration assay.

Results: Average diameter and particle concentration of the olive flounder plasma derived exosomes were 151.82±9.17 nm and $6.31x10^{10}$ particle/mL, respectively. TEM analysis showed characteristic appearance of the exosomes with sharp membrane margin and unique cup shape morphology. Immunoblot analysis showed the presence of exosome marker proteins and absence of plasma proteins. Exosomes had no cytotoxicity for both FHM and raw 264.7 cells up to 100 µg/mL and no toxicity related mortality and reactive oxygen species generation in zebrafish larvae up to 400 µg/mL. Induction of ant-inflammatory cytokines, and inhibition of pro-inflammatory cytokines and pro-apoptotic genes were observed. *In vitro*-scratch wound healing and *in vivo*-zebrafish fin regeneration assays showed significantly ($p<0.05$) enhanced wound healing and tissue regeneration activity at 50 and 100 µg/mL of exosomes.

Conclusion: This study provides evidence of the presence of typical exosomes in olive flounder plasma and high level of wound healing activity *in vitro* and *in vivo* with no toxicity.

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