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Original article

# Effect of genistein on regenerative angiogenesis using zebrafish as model organism

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ABSTRACT

We examined the effects of phytoestrogen genistein on zebrafish caudal fin regeneration and found that genistein could inhibit fin generation in μM concentration. Fish were injected with varying concentrations (10–100 μM) of genistein for 5 days after caudal fin amputation, and regeneration of the fin was evaluated by analyzing caudal fin length and newly formed vascular region. Regeneration of amputated fins was inhibited or delayed to the maximum of 64.7% in dorsal region, 63.35% in cleft region and 66.54% in ventral region after genistein treatment unlike the control that completely regenerated their fins after 5 days post-amputation (DPA). PCR data showed a clear reduction in vascular endothelial growth factor (VEGF) expression on exposure to genistein while a complete inhibition was observed with sunitinib (SU 11652) an inhibitor of VEGF signaling. The ability of genistein to inhibit regenerative angiogenesis of caudal fin probably by down regulating VEGF, the key player of angiogenesis and the results obtained with SU 11652 is suggestive of the involvement of VEGF signaling during regeneration. These results demonstrate that zebrafish could be a good model in elucidating molecular mechanisms that are responsible for fin regeneration.



## 1. Introduction

In the field of tissue engineering and development, all organisms withhold a biological response to tissue damage and wound healing [1]. Distinct comparative models are source to study, zebrafish takes one of the lead roles to study angiogenesis in parallel with molecular mechanisms associated with human diseases [2] We used zebrafish as an animal model to study angiogenesis, in fact it shares a similar ancestor property with human [3]. Regeneration of caudal fins is fast and robust in adult, and regenerates after fourteen days of amputation [4,5]. From epidermal covering to blastema formation and requiring structures will pave way to fin regeneration after amputation [6–10]. Significance of VEGF-A (165) and its receptors rules the proliferation of endothelial cells in regeneration mechanism regulating angiogenesis [11,12].

Extensive research over years has indicated that phytochemicals including genistein, resveratrol, quercitin, apigenin, naringenin, thymoqiunone, gingerol have medicinal properties and are known to possess therapeutic importance as dietary compounds. Most of these lack toxicity, which backup the safety measures in daily consumption. Isoflavones belong to this group of phytochemicals commonly consumed in food, found enriched in soy and other legumes and known to posses strong anti oxidant, anticancer properties. With its prospective, the effect of isoflavone genistein on regenerative angiogenesis is studied using zebrafish as a model organism.

Genistein is a dietary plant compound derived from soy and known to possess estrogenic and anti estrogenic, exhibiting antineoplastic activity, anti-angiogenic and anticancer activity [13,14]. The anti-angiogenic activity of genistein was positively reported in zebrafish larvae at 2 μM and suggested to bind with VEGF receptors, suppressing VEGF signaling [15]. Only a few plant compounds such as quercitin have been evaluated for its potential to inhibit regenerative angiogenesis during zebrafish tail fin regeneration. In the present study attempts have been made to study the efficacy of genistein during tail fin regeneration using zebrafish adult model and the results obtained were compared with that of SU 11652, a potential inhibitor of VEGF signaling pathway mainly to evaluate the role of VEGF during fin regeneration.

Here we demonstrate that genistein in the range of 10–100 μM inhibits regenerative angiogenesis by down regulating VEGF and suppressing the formation of blood vessels, vessel densities, its vasculature, and fin length. Comparison of the results obtained with genistein with that of SU 11652 suggests that VEGF signaling might play an important role during tail fin regeneration.

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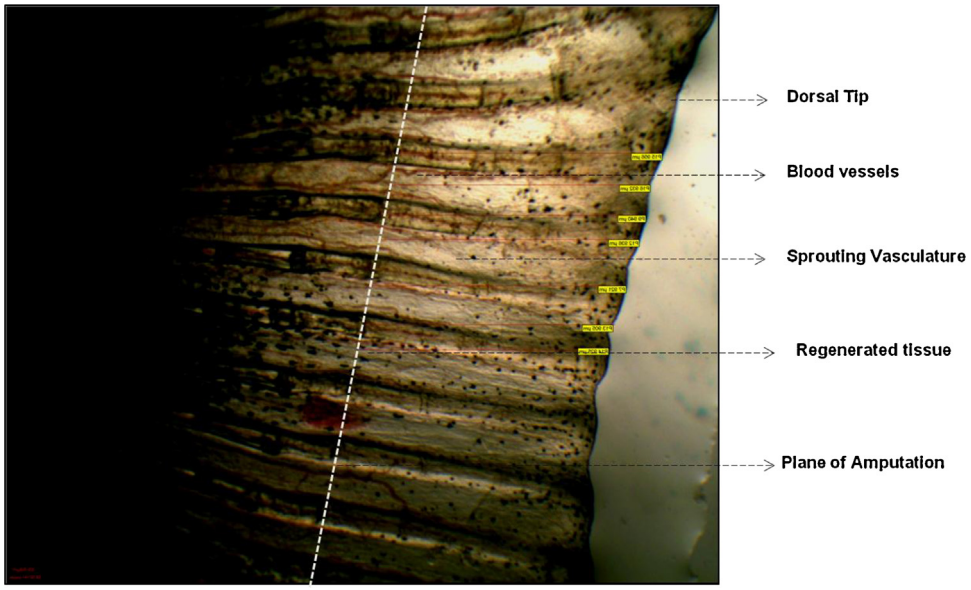


**Fig. 1.** Area of regeneration: zebrafish control partially amputated caudal fin showing different region of vasculature from the plane of amputation.

## 2. Materials and methods

### 2.1. Plastic wares, glass wares

Fish tanks, adult wild type zebrafish, pasteur pipettes, glass slides, light microscope, digital camera, scalpel, razor blade. All plastic and glass wares used in this study were obtained from Tarsons (P) Ltd.

### 2.2. Chemicals and reagents

Genistein, dimethyl sulfoxide (DMSO), SU 11652 were purchased from Sigma Aldrich Chemicals. Pvt. Ltd. (USA). Tri Reagent was purchased from Invitrogen, Tricaine MS-222 from MP Bio Medicals. Reverse transcription was performed using random primers (Promega), riboblock (Thermo Scientific), reverse transcriptase (MMuLV), reverse transcriptase buffer (MMuLV), DNTPs

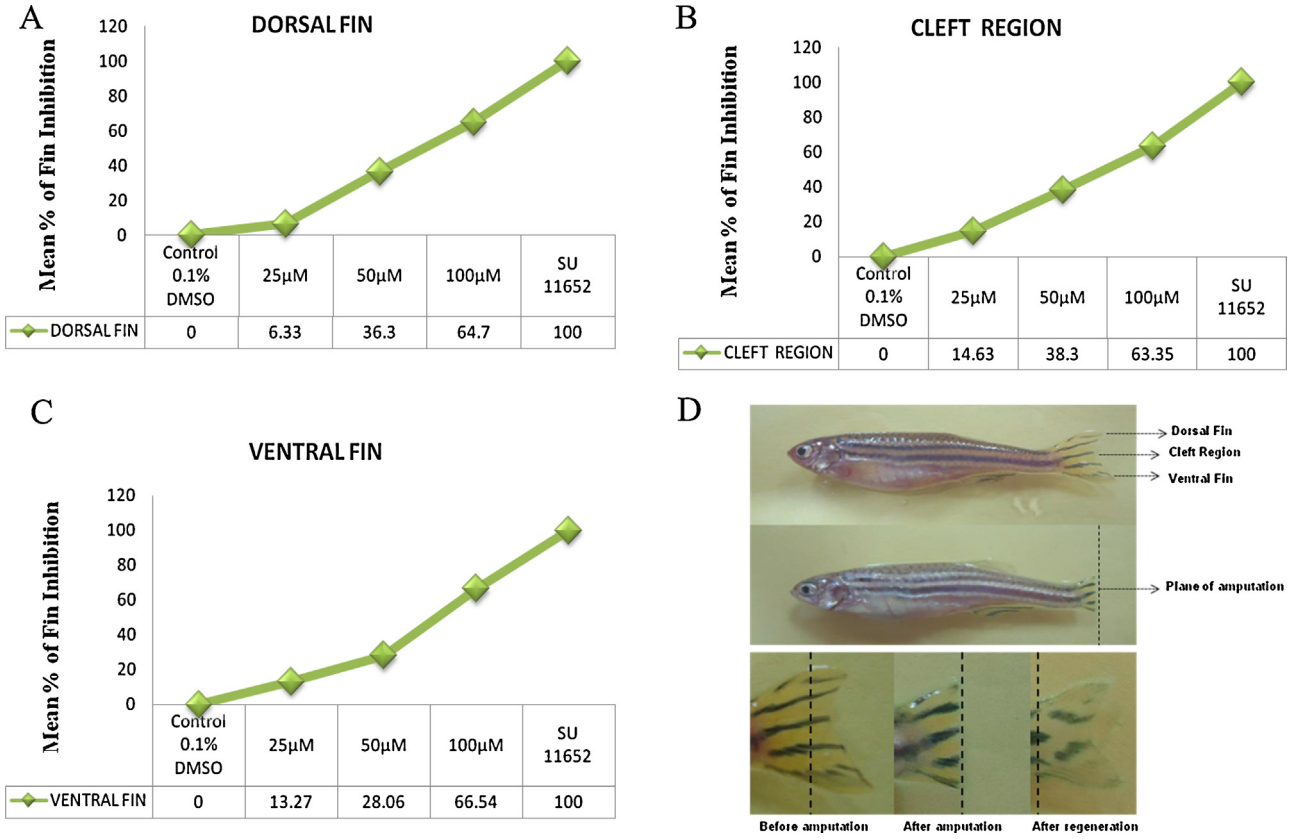


**Fig. 2.** A–C. Inhibition of zebrafish fin when treated with genistein in dose-dependent behavior. Adult zebrafish were injected (ip) with 25 µM, 50 µM, 100 µM genistein and 1 µM SU 11652, and their caudal fins were partially amputated. The percentage fin regrowth was calculated after 5 days of amputation. Mean, SD, $n = 5$. D. Caudal fin showing different area of the region exhibiting amputation site and regenerated tissue.