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(RESEARCH ARTICLE)

# The effect of resveratrol and alcohol on zebrafish caudal fin regeneration

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## Abstract

**Introduction:** Wine, an alcoholic beverage derived from the fermentation of fruit juices, typically grapes, has been a popular drink since ancient times. Resveratrol, a polyphenol found in grapes, is associated with growth inhibition. There are conflicting reports on the overall health benefits of regular wine consumption, with some sources suggesting positive effects on growth, while others claim inhibitory effects. This study investigates the effects of red wine, grape juice, and resveratrol on growth and wound healing using adult zebrafish.

**Method:** The study assessed growth through the regeneration of the caudal fin (CF) in adult zebrafish. The CF was amputated, and the fish were subjected to various treatments: different concentrations of alcohol, grape juice (GJ), resveratrol, red wine, and combinations of alcohol with grape juice (A+GJ) or resveratrol (A+RV). Treatments were administered for 1 hour, three times daily, with a 1-hour recovery period between treatments, over 14 days. CF growth was measured using Fiji ImageJ.

**Results:** Grape juice, alcohol, and red wine treatments resulted in significant, concentration-dependent reductions in CF regeneration compared to untreated fish. The A+GJ combination showed increased regeneration compared to GJ alone, but regeneration was still lower than in untreated fish. Resveratrol-treated fish exhibited higher CF regeneration compared to the control. High concentrations of resveratrol in A+RV treatments caused significant toxicity, while lower doses led to reduced CF regeneration.

**Conclusion:** The growth inhibition observed with red wine and GJ treatments may not be solely due to resveratrol but rather the interaction of resveratrol with other components of GJ and/or alcohol.

**Keywords:** Growth; Red wine; Grapes; Alcohol; Zebrafish

## 1. Introduction

Wine has been one of the most popular beverages since ancient times. It is a fermented fruit juice, most commonly made from grapes, with alcohol levels ranging from 5-16% vol/vol. Red wine, in particular, is widely consumed and is reputed to offer numerous health benefits when consumed regularly. These benefits are often attributed to the various phenolic compounds present in wine. Studies have shown that these compounds can favorably influence multiple biochemical systems, such as increasing high-density lipoprotein cholesterol, enhancing antioxidant activity, decreasing platelet aggregation and endothelial adhesion, suppressing cancer cell growth, and promoting nitric oxide production [1,2]. However, these compounds can also negatively impact several biochemical processes, including wound healing and tissue repair, angiogenesis, and the inhibition of some cytochrome P450 enzymes [3, 4].

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Red wine's effect on wound healing is often attributed to the alcohol it contains or the polyphenol resveratrol. Alcohol has been shown to impair the wound healing process by negatively impacting re-epithelialization, angiogenesis, collagen production, and wound closure [5]. It causes major reductions in wound angiogenesis by decreasing the expression of vascular endothelial growth factor (VEGF) receptors and reducing nuclear expression of HIF-1alpha in endothelial cells [6,7]. Resveratrol, also known as 3,4,5-trihydroxystilbene, is a naturally occurring polyphenol found in grape skins that accumulates during the fermentation process of wine. Like alcohol, resveratrol inhibits wound healing by impeding cellular migration, inhibiting endothelial cell growth, and affecting various MAP kinases [8,9]. Resveratrol also inhibits angiogenesis by binding it to VEGF and impeding their action [10].

This study aims to determine the effect of regular red wine consumption on wound healing and tissue growth by utilizing the regenerative nature of the zebrafish caudal fin. The zebrafish caudal fin is highly regenerative, with the ability to fully regenerate approximately 14 days after damage [11]. The fin comprises several segmented bony structures called bony rays and mesenchymal tissue in between, enclosed in the epidermis. Each bony ray consists of two concave hemi-rays that define an inner space filled with intra-ray mesenchymal cells. Blood vessels and nerve axons are found in both intra- and inter-ray tissues. Bony rays are produced and maintained by osteoblasts, skeletogenic cells that secrete bone matrix [12]. The caudal fin regenerates rapidly and contains many different tissue types, making it suitable for the analysis of wound healing.

In this study, amputated zebrafish were subjected to different treatments: alcohol, grape juice, red wine, resveratrol, and combinations of resveratrol or grape juice with alcohol. Grape juice, considered the non-alcoholic component of red wine, was combined with alcohol to reconstitute wine. This approach was used to assess whether the combination of alcohol and grape juice has a different impact on wound healing compared to red wine. Additionally, this study aims to determine if the inhibition of wound healing observed with red wine is due to resveratrol or the alcohol component of wine.

## 2. Methods and Materials

### 2.1. Material

Wild type zebrafish, white overproof rum (63%), de-chlorinated water, Hardy's red wine (16.5%), Andonstar microscopic webcam, glass plate, hot plate, HD720 webcam, API Melafix antibacterial solution, API Pimafix (antifungal solution), bluelab combo meter (pH meter), ocean sea salt, 1% phenoxyethanol, scalpel, sodium hydroxide, vinegar, laptop running iSpy software and Fiji ImageJ software, Welch's 100% concord grape juice, Nature's Way 37.5 mg resveratrol capsules.

### 2.2. 1% 2-Phenoxyethanol Preparation

A 1% stock solution of 2-phenoxyethanol was prepared by adding 5 mL of 100% pure 2-phenoxyethanol to 500 mL of distilled water. Then, 6 mL of this solution was added to 400 mL of fish water to serve as an anesthetic for the fish.

### 2.3. Fish Selection and Maintenance

Healthy adult zebrafish, approximately 5-6 cm in length, were selected from storage tanks and divided into seven different treatment groups, each containing 18 fish. Each group was further divided into three sub-groups of six fish each. The sub-groups were housed in separate 4L fish tanks under optimal conditions as suggested by Westerfield, 2019 [13], with a temperature of 28.5 °C and alternate 14-hour light and 10-hour dark cycles. Continuous aeration was provided using an air pump, and the fish were fed three times daily. Water quality was maintained by replacing 30% of the tank water daily with dechlorinated tap water.

### 2.4. Resveratrol Preparation

Stock solutions of resveratrol were prepared daily by dissolving the contents of a Nature's Way resveratrol capsule in 100 mL of water. The mixture was heated to aid solubility and then cooled before use.

### 2.5. Caudal Fin Amputation and Regeneration

Fishes were individually removed from their tanks and placed in a tank containing 0.015% phenoxyethanol anesthetic solution. Anesthesia was confirmed by loss of tail reflex. Each fish was then placed on a petri dish, dried, and its uncut caudal fin was photographed using an Andonstar microscopic webcam. Approximately half of the caudal fin was cut with a scalpel, and a photo was taken immediately after. The fish were returned to their housing tanks for 14 days. Post 14-day recovery, the fish were re-anesthetized, and photos of the regenerated caudal fins were taken. The area of the

fin before amputation and after regeneration as shown in Figure 1A and 1B, was measured using Fiji ImageJ software, and comparisons were made to determine regeneration efficiency.

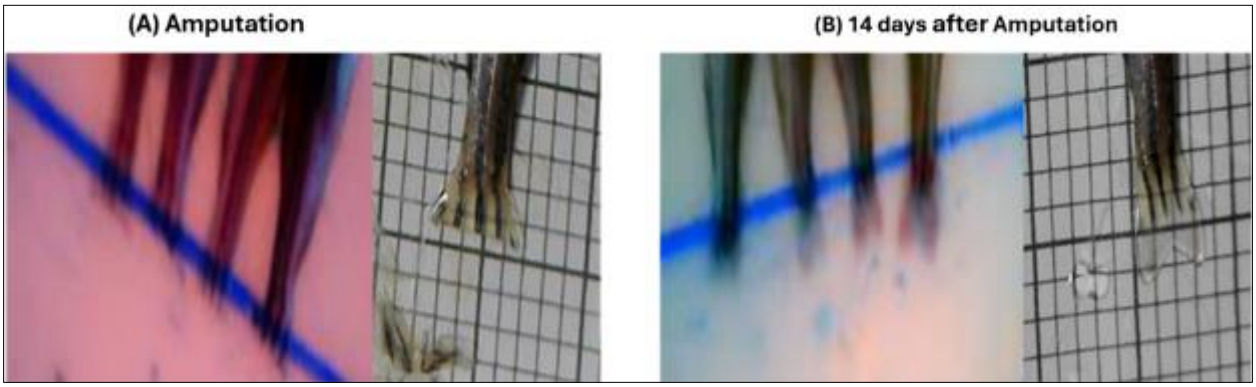


**Figure 1** The zebrafish caudal fin and its regeneration after amputation

### 2.6. Drug Treatment

Solutions of red wine (0, 0.05, 1, and 1.5%), grape juice (0, 0.05, 1, and 1.5%), alcohol (0, 0.5, 1, and 1.5%), and resveratrol (0.3, 0.6, and 0.9 mg/L) were prepared by diluting specific volumes of red wine, grape juice, resveratrol, or 63% alcohol solution, respectively, with fish water to a final volume of 500 mL. Amputated fish were treated three times daily for 1 hour with a 1–2-hour break between treatments for 14 days. Fish were placed in 500 mL of the respective drug treatment solutions.

An alcohol concentration with a low mortality rate and minimal impact on regeneration was selected and combined with different concentrations of grape juice or resveratrol to observe combined effects. Amputated fish were exposed to these treatments in a similar manner as single drug treatments.

### 2.7. Statistical Analysis

One-way ANOVA with Tukey's multiple comparison tests was performed on the data sets using GraphPad Prism version 6 statistical software. P-values <0.05 were considered statistically significant. Data were expressed as mean ± standard deviation or average percentage ± standard deviation [14].

## 3. Results

A comparison of decreases in caudal fin regeneration with increasing alcohol and red wine concentrations, as depicted in Figure 2a. Alcohol concentrations above 0.5% showed significant reductions in regeneration (1.0% and 1.5%, $p < 0.001$) compared to the control. Higher alcohol concentrations also exhibited toxicity. Red wine treatments resulted in significant reductions in caudal fin regeneration compared to both the control ($p < 0.001$) and alcohol-only treatments ($p < 0.001$), as shown in Figure 2 (A). Grape juice treatments alone also reduced caudal fin regeneration, with significant reductions in growth compared to the control ($p < 0.001$), as illustrated in Figure 2 (B). However, no significant differences were observed between grape juice and red wine treatments.

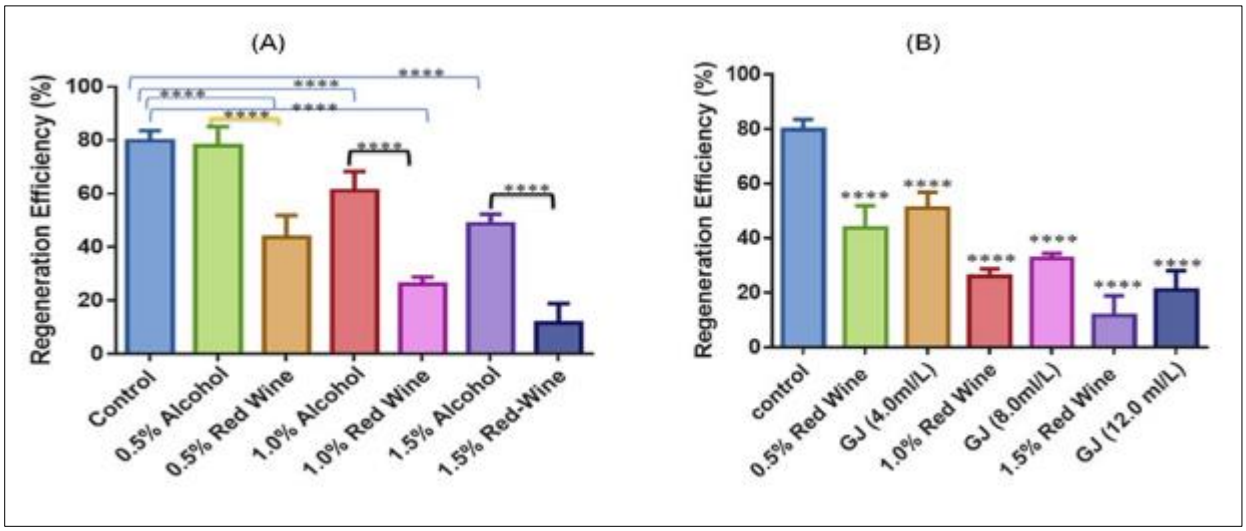


**Figure 2** Comparison of Zebrafish caudal fin regeneration under different treatment conditions [(A) control, alcohol and red wine] and [(B) control, red wine and grape juice (GJ)]

Combining grape juice with alcohol revealed changes in caudal fin regeneration. When 0.5% alcohol was combined with 8 mL or 12 mL grape juice, significant reductions in tissue growth were observed ($p < 0.001$) compared to the control or alcohol-only treatments, similar to grape juice-only treatments. However, combining 0.5% alcohol with a lower dose of grape juice (4 mL) significantly increased tissue growth ($p < 0.001$) compared to grape juice-only treatments, comparable to the control and alcohol-only treatments (Figure 3).

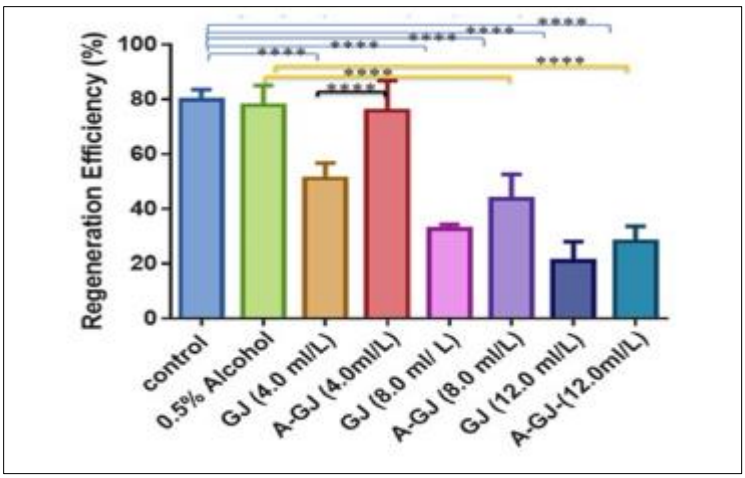


**Figure 3** Comparison of Zebrafish caudal fin regeneration 0.5% alcohol (v/v) reconstituted with different concentration of grape juice (GJ)

When resveratrol doses similar to those in wine treatments were used, no significant reductions in tissue growth were observed. However, combining these resveratrol doses with 0.5% alcohol resulted in high toxicity, with fish displaying seizure-like behavior before death. Lower doses of resveratrol combined with alcohol showed concentration-dependent decreases in tissue growth, which were significant ($p < 0.001$) compared to resveratrol-only treatments or the control Figure 4.

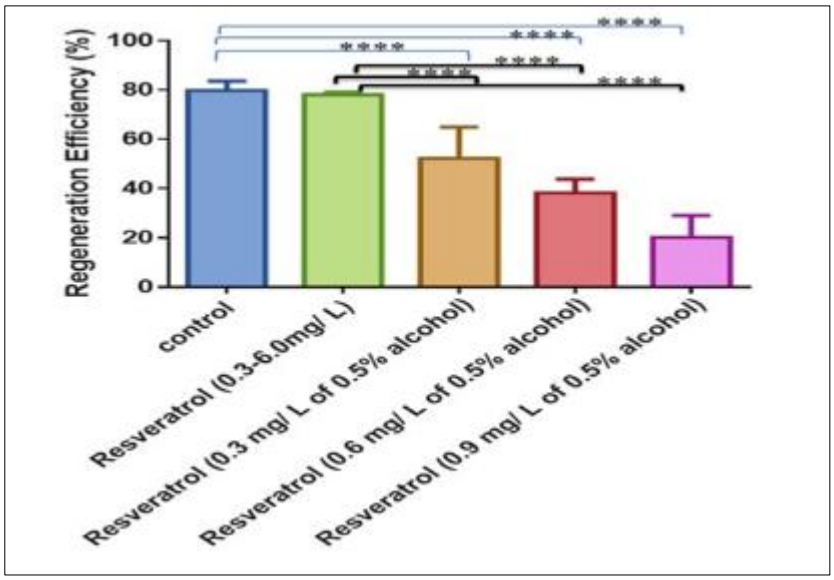


**Figure 4** Comparison of Zebrafish caudal fin regeneration 0.5% alcohol (v/v) reconstituted with different concentration of Resveratrol

## 4. Discussion

This study demonstrates that alcohol concentrations above 0.5% significantly impair tissue regeneration in zebrafish, aligning with recent findings on alcohol's negative impact on wound healing [5, 15]. The toxicity observed at higher alcohol concentrations reinforces the detrimental effects of alcohol on cellular processes [16].

Red wine treatments showed significant reductions in regeneration compared to control and alcohol-only treatments, indicating that components other than alcohol in red wine contribute to this effect. Grape juice alone also reduced regeneration, suggesting that compounds in grape juice are responsible for the observed inhibitory effects. This aligns with recent studies highlighting the complex interactions of polyphenols and other organic compounds in grape juice [17].

Interestingly, low doses of alcohol combined with grape juice mitigated the inhibitory effects of grape juice alone, suggesting a protective role of alcohol against grape juice-induced growth reduction. This finding aligns with the concept that moderate alcohol consumption can have varying effects depending on the context and combination with other compounds [18].

Resveratrol alone did not significantly affect regeneration, but its combination with alcohol led to severe toxicity and reduced growth, indicating a detrimental interaction. This finding supports recent research showing that resveratrol can have complex effects on cellular processes, especially when combined with other substances [19].

## 5. Conclusion

Regular exposure to red wine leads to reduced tissue growth and repair in zebrafish. These reductions are not solely due to the alcohol or resveratrol present in wine but are likely due to the interaction of resveratrol with other organic compounds in grape juice. The alcoholic component of wine may offer some protective effect against grape juice-induced growth reduction at low doses. However, the combination of alcohol and resveratrol is particularly detrimental to growth and overall health.

## Compliance with ethical standards

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*Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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