

Regenerative effects of turmeric and alpha-lipoic acid in *Lumbriculus variegatus*

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SUMMARY

Clinicians face challenges in healing avascular tissues with low cell density, such as those in joints, where the healing process following injury is slow. To investigate potential natural compounds for tissue repair and their effect on physiological activity, we examined the effects of turmeric and alpha-lipoic acid (ALA) on segment regeneration and cardiovascular pulse rate, using *Lumbriculus variegatus*, known as the California blackworm. We hypothesized that these naturally derived compounds would enhance regenerative outcomes and that pulse rate measurements could help determine whether regeneration was associated with broader changes in physiological activity. The worms were assigned to a control group, a turmeric treatment group, or an ALA treatment group. After an amputation in the anterior region, the worms were measured by the regrowth of segments and pulse rate at four different time points. Worms treated with turmeric had a significantly longer segment length than the control group ($p < 0.05$), whereas worms treated with ALA did not show significant results ($p > 0.05$). Compared to ALA, turmeric treatment resulted in a significantly greater degree of segment regeneration ($p < 0.05$). Turmeric resulted in greater regenerative outcomes compared to both the control and ALA groups, while fluctuations in pulse rate indicated no clear relationship to regenerative outcomes ($p > 0.05$). Turmeric-derived curcuminoids, the primary bioactive components of turmeric, demonstrated regenerative effects in a simple invertebrate model and may inspire future studies in mammals and humans.

INTRODUCTION

The healing of damaged tissues is a slow process that often reduces the time that athletes spend on their activities. In contrast to organisms capable of whole-limb or organ regeneration, humans rely on adult stem cells that enable localized tissue repair and maintenance but lack the capacity for large-scale regeneration, such as complete organs or limbs (1). More specifically, joint injuries to the articular cartilage are challenging to treat due to the cartilage's limited intrinsic healing capacity (2). The meniscus is a structure that helps distribute pressure in the knee joint, and in the context of healing lateral meniscus tears of the knee, the meniscus's avascular nature and low fibrochondrocyte density result in slow recovery (3). Two-thirds of the lateral meniscus, the

white zone, is avascular and obtains its nutrition through passive diffusion (4). Conventional therapies such as physical therapy, medication, and surgery often provide only partial or temporary relief rather than complete tissue restoration (2). Surgical interventions, such as partial meniscectomy or meniscus repair, lead to incomplete healing, and many patients eventually develop degenerative complications, including early-onset osteoarthritis (3). As a result, healing of the meniscus is limited without intervention, and routine clinical procedures such as partial meniscectomy generally do not prevent the progressive onset of osteoarthritis (4). Diffusion is passive in acellular tissues, which limits the delivery of nutrients and therapeutic compounds (2, 3). To address this barrier, tissue engineering strategies explore natural agents such as turmeric and alpha-lipoic acid (ALA), which have demonstrated antioxidant and anti-inflammatory properties that may support tissue repair (5).

Turmeric (*Curcuma longa*) is a rhizomatous plant with traditional medicinal uses and a growing body of clinical and preclinical studies supporting its use in medicinal practices (5). Turmeric contains bioactive polyphenolic compounds, known as curcuminoids (e.g., curcumin, dimethoxycurcumin, and bisdemethoxycurcumin), that have been researched in nutraceuticals and as functional superfoods (5). Research suggests that curcuminoids have various cellular activities that help reduce inflammation and tumor formation (5). Curcuminoids inhibit the cytokines IL-1 and TNF, reducing reactive oxygen species (ROS)-induced damage and suppressing NF- κ B activity, thereby decreasing inflammation, cell death, and diseases associated with oxidative damage (6). Turmeric has also been shown to impact cardiovascular physiology (5). Certain nutraceutical compounds, including curcumin, may reduce blood pressure, limit free radical damage to cardiac tissue, and support vascular function (7). Additionally, in heart injury models, curcumin has been shown to improve heart function through its antioxidant and anti-inflammatory properties (8). The healing process may also be aided by mechanisms involving the circulatory system, which supports tissue recovery (2). It is believed that these improvements in the circulatory system may involve cellular and antioxidant mechanisms involved in tissue and cell regeneration (6).

Alpha-lipoic acid (ALA) is a naturally occurring compound that influences mitochondrial metabolism and has antioxidant properties (9). It is a free-radical scavenger that helps regenerate endogenous antioxidants, including glutathione and vitamins C and E, and stabilizes membranes under oxidative stress (9, 10). When ALA is absorbed and distributed to tissues, it acts in both aqueous and lipid environments within cells (11). Physiological benefits of ALA may contribute

to cardiovascular stability and create environments favorable for tissue repair and recovery (10). As a cofactor for the mitochondrial enzyme complexes pyruvate dehydrogenase and α -ketoglutarate dehydrogenase, ALA may restore redox balance and protect mitochondria from dysfunctions related to injuries or diseases (10). In addition to its role in mitochondrial metabolism, ALA has been shown to attenuate cellular damage associated with oxidative stress and inflammation (12). In human models, ALA has been shown to reduce intracellular ROS levels, implying protective effects against oxidative injury at the cellular level (12). Regarding tissue recovery, ALA has also been shown to promote epithelial injury repair by modulating mitogen-activated protein kinase (MAPK) signaling pathways, which regulate cell survival and regeneration following damage (13). ALA's antioxidant activity and regulation of redox-sensitive signaling pathways contribute to physiologically favorable cellular environments post-injury. ALA can also modulate redox-sensitive transcription factors, including NF- κ B, leading to reduced expression of pro-inflammatory cytokines and attenuation of inflammatory signaling following cellular stress (14). Through its antioxidant and anti-inflammatory properties, ALA may support recovery by limiting secondary damage associated with oxidative and inflammatory responses (14).

While prior research shows that turmeric and ALA have anti-inflammatory properties, little is known about their ability to support tissue repair. In order to study these properties that may induce tissue repair and improve physiological function, a relevant experimental model was needed to evaluate these effects. *Lumbriculus variegatus*, a freshwater oligochaete known as the California blackworm, was selected because of its quantifiable regenerative responses following injury. After transverse amputation, *Lumbriculus variegatus* regenerates lost anterior or posterior segments through wound healing, blastema formation, cell proliferation, and directed cell migration (15). Clearly defined regenerative stages allow it to be tracked quantitatively, making it well-established for investigating mechanisms of tissue repair at the cellular level (15). Studies have shown that successful regeneration in *Lumbriculus variegatus* involves redox signaling as an essential

component of regeneration start and stop signals (16). Since both turmeric-derived curcuminoids and ALA exert biological effects through antioxidant activity and by modulating redox pathways, both supplements are viable for study. In addition to its regenerative capacity, *Lumbriculus variegatus* is well-suited for studying physiological responses, such as pulse rate, to these bioactive compounds (17). Although dorsal blood vessel pulse rate does not directly measure regeneration, it acts as a secondary physiological endpoint when evaluated alongside regenerative capacity and oxygen consumption in *Lumbriculus variegatus* (18). Since *Lumbriculus variegatus* is diffusion-limited in its nutrient transport, it serves as a relevant model for avascular human tissues, such as the meniscus, where nutrients are similarly delivered through diffusion within the extracellular matrix, providing a relevant system to test how antioxidant compounds can influence both tissue repair through regenerated segments and physiological function, influencing the availability of nutrients to aid repair processes. Similarly, diffusion-limited conditions are found in human meniscal tissue, where turmeric stimulates biological pathways to modulate inflammation, oxidative stress and cellular signaling, facilitating passive diffusion of nutrients in the absence of a direct blood supply, supporting its use as a model to investigate how anti-inflammatory and antioxidative compounds can influence repair and physiological function in diffusion-limited systems (19).

We hypothesized that turmeric and ALA influence tissue repair and physiological function in *Lumbriculus variegatus*, as measured by segment regrowth and pulse rate. We measured segment regeneration and pulse rate in *Lumbriculus variegatus* post-anterior amputation, supplemented with exposure to turmeric or ALA to evaluate how these compounds affect regenerative capacity and physiological activity. Our results showed that turmeric treatment resulted in a significant level of segment regeneration compared to both ALA and the control, while ALA did not produce any significant effect on regrowth. However, we did not find any statistical difference in pulse rates. Together, these findings support the use of simple invertebrate models, like *Lumbriculus variegatus*, to investigate tissue regeneration in diffusion-limited systems,

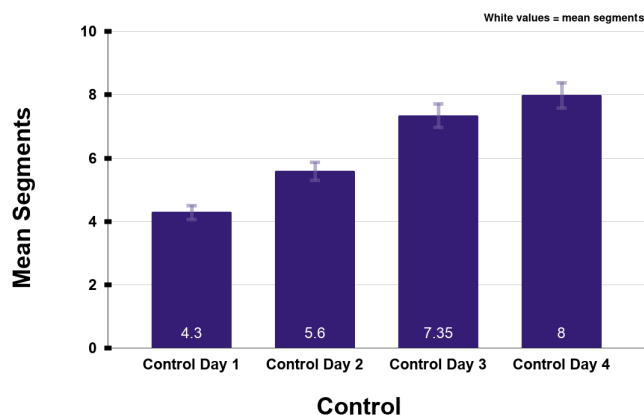


Figure 1: Effect of control on segment regeneration in *Lumbriculus variegatus*. The mean number of regenerated segments was measured over four treatment days post-anterior amputation and is reported on the graph in white. Newly formed segments were counted every 24 hours using a digital microscope. Data represent mean $\pm$ standard deviation (SD) ($n = 20$ worms). Worms were fed un-supplemented fish food.

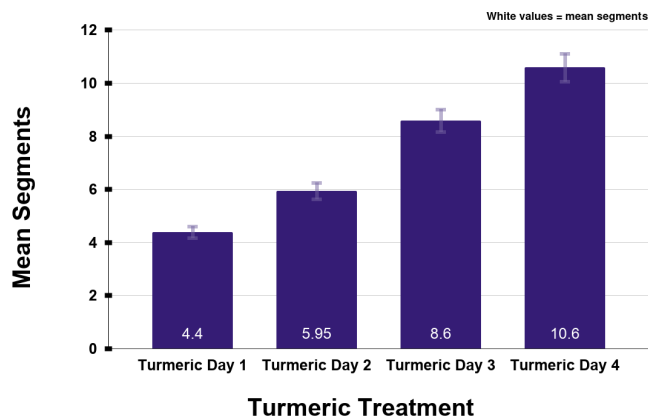


Figure 2: Effect of Turmeric treatment in *Lumbriculus variegatus*. The mean number of regenerated segments was measured post-anterior amputation in worms treated with turmeric-supplemented fish food (0.1g per 10 g food) and is reported on the graph in white. Data represent mean $\pm$ SD ($n = 16$ worms).

providing insights into similar processes in avascular human tissues such as the meniscus. While the human meniscus, particularly the inner avascular 'white-white' zone, has limited spontaneous healing capacity, the study does not claim regeneration of the human meniscus; it investigates whether compounds such as turmeric and ALA enhance regenerative biology in an experimental model that may inform future research.

RESULTS

To determine the effects of turmeric and ALA on the regeneration and physiological activity of *Lumbriculus variegatus*, we administered each compound at a dose of 0.1 g per 10 g of fish food for 2 weeks following anterior amputation. We measured segment length and heart rate every 24 hours, with morphological observations recorded concurrently.

Segment length

The control group (no treatment administered) worms established a baseline for subsequent segment regeneration and physiological assessment (Figure 1). We assessed regeneration as the change in the mean number of regenerated segments compared to the first post-amputation measurement. In the control group, individual regenerated segment counts ranged from 3 to 11 across all trials. Compared to the control, the turmeric treatment group showed significantly higher levels of segment regeneration across all 16 trials (Figure 2; $p < 0.05$). However, ALA-treated worms showed lower regenerated segments relative to the control group, with variation between trials, but this difference was not statistically significant (Figure 3; $p > 0.05$). A direct comparison between the turmeric and ALA treatment groups showed that turmeric produced a significantly greater response than ALA (Figures 2 and 3; $p < 0.05$).

Heart rate

We measured heart rate in all experimental groups, and controls maintained relatively stable pulse rates across all trials, with measurements between 12 to 20 beats per minute (bpm) on each of the four observation days (Figure 4). Turmeric-treated worms showed modest increases in pulse rate; however, these changes were not statistically significant (Figure 5; $p > 0.05$). ALA-treated worms exhibited variable pulse rates with no discernible pattern, indicating no significant effect (Figure 6; $p > 0.05$). A direct comparison between the turmeric and ALA treatment groups revealed no significant difference in pulse rate response (Figures 5 and 6; $p > 0.05$).

Post-amputation morphology

Microscopic images captured the healing outcomes following anterior amputation of the clitellum. Worms failing to recover, as pre-determined by criteria like poor health, including loss of motility 24 hours post-amputation, visible tissue necrosis, or death, were not included in the study (Figure 7). Worms that successfully healed after amputation showed uniform segmental regeneration and intact tissues (Figure 8).

DISCUSSION

The results of the experiments suggest that turmeric may enhance tissue regeneration in *Lumbriculus variegatus*, as

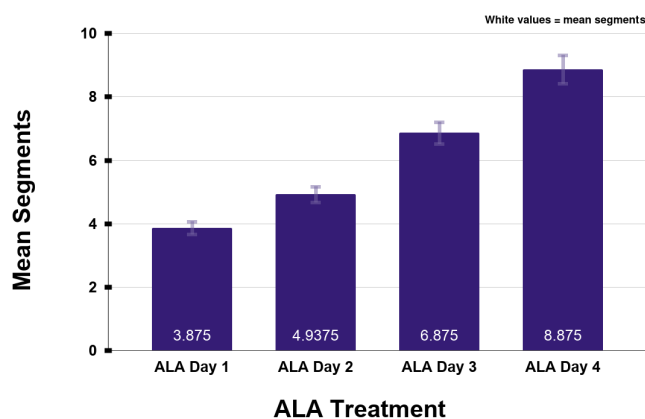


Figure 3: Effect of alpha-lipoic acid (ALA) treatment in *Lumbriculus variegatus*. The mean number of regenerated segments was measured post-anterior amputation in worms treated with ALA-supplemented fish food (0.1 g per 10 g food). Data represent mean $\pm$ SD ($n = 16$ worms).

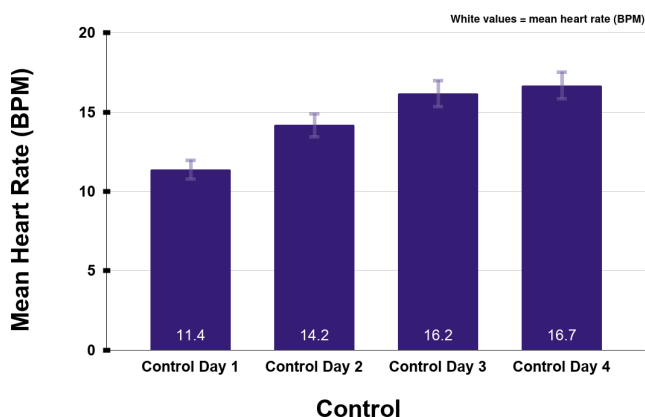


Figure 4: Effect of control on heart rate in *Lumbriculus variegatus*. The mean heart rate was measured over four treatment days post-anterior amputation and is reported on the graph in white. Dorsal vessel contractions were counted over a 30-second interval and averaged across three trials per worm. Data represent mean $\pm$ SD ($n = 20$ worms). Worms were fed un-supplemented fish food.

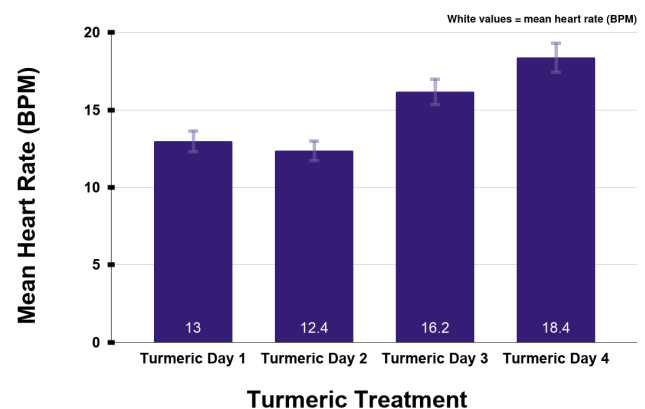


Figure 5: Effect of turmeric treatment on heart rate in *Lumbriculus variegatus*. The mean heart rate was measured post-anterior amputation in worms treated with turmeric-supplemented fish food (0.1 g per 10 g food) and is reported on the graph in white. Dorsal vessel contractions were counted over a 30-second interval and averaged across three trials per worm. Data represent mean $\pm$ SD ($n = 16$ worms).

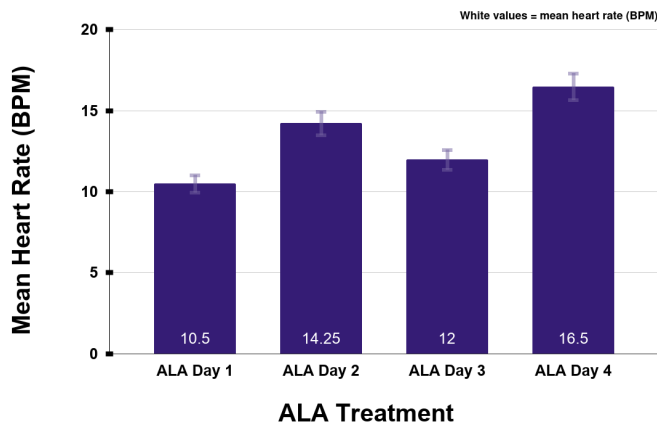


Figure 6: Effect of alpha-lipoic acid (ALA) treatment on heart rate in *Lumbriculus variegatus*. The mean heart rate was measured post-anterior amputation in worms treated with ALA-supplemented fish food (0.1 g per 10 g food) and is reported on the graph in white. Dorsal vessel contractions were counted over a 30-second interval and averaged across three trials per worm. Data represent mean $\pm$ SD ($n = 16$ worms).

reflected by increased segmental regeneration. Worms treated with turmeric showed greater mean segmental regeneration compared to control worms (Figure 2; $p < 0.05$). This supports the hypothesis that turmeric would enhance mean segmental regeneration, which may have contributed to improving tissue repair through antioxidant and anti-inflammatory properties, although these mechanisms were not directly tested in the study (6). Turmeric reduces ROS-induced damage while suppressing inflammatory signaling pathways, facilitating these effects (5, 6). Also, turmeric supports vascular and cellular function, contributing to mechanisms of action associated with the limitation of inflammation and promotion of cell division (7, 8). These results are relevant to avascular human tissues such as the meniscus, where similar nutrient delivery mechanisms allow antioxidant compounds like turmeric to improve healing outcomes (2, 4).

However, our results did not support the hypothesis that ALA would enhance tissue regeneration as the high variability in growth rates within this treatment group yielded statistically nonsignificant results compared to the control (Figure 3; $p > 0.05$). Consequently, we expected the ALA group to show greater regenerative improvement because ALA has been shown to reduce oxidative stress and enhance endothelial function, creating a suitable environment for tissue repair (11). ALA has been shown to reduce oxidative stress and inflammatory signaling while supporting cardiovascular and endothelial function, which contribute to improved circulation (10-12, 14). Nonetheless, ALA, which works at the cellular and metabolic levels, may not influence the type of large-scale regeneration assessed in this study.

We recorded pulse rate as a physiological indicator, with variations in heart rate reflecting changes in circulation and metabolic activity. Alterations in circulation following injury may support tissue repair by facilitating oxygen and nutrient delivery, rather than by directly influencing heart rate (1, 5, 10). There were no significant changes in the pulse rate across any of the treatment groups, suggesting that regenerative outcomes were not associated with measurable changes in pulse rate (Figures 4, 5, and 6; $p > 0.05$). The

effects may instead stem from signaling at the damage site or from a metabolic change that does not affect whole-organism physiology. More robust measures taken over a longer period in future studies may reveal subtle effects not observed here. Anterior amputation of the clitellum caused irrecoverable distress in some specimens, and microscopic observations supported these findings. Some worms showed signs of tissue necrosis, irregular segment spacing, and loss of motility, and as a result, were not included in the study. However, the specimens that successfully healed during treatment days with observable signs of quantitative regeneration and intact tissues were included in the quantitative analysis.

Our study had several limitations, including the lack of a positive control, which limited direct comparisons to established supplements. Additionally, single dosage treatments and a short treatment duration may have limited the analysis of subtle or delayed effects. It is possible that the effects of ALA have a delayed onset, so increasing the duration and dosage of treatment may yield statistically significant results not observed here.

Based on our results, it would be important to test turmeric in vertebrate or mammalian models for its translational potential. *Lumbriculus variegatus* is a viable regeneration model with conserved processes like cell proliferation, migration, and redox signaling (15, 16). However, *Lumbriculus variegatus* is a simpler organism than humans, and its diffusion-based physiology allows tissue repair to be studied in isolated environments. Further investigation into its role in tissue repair and its possible relevance to diffusion-limited human tissues, such as the meniscus, is needed.

MATERIALS AND METHODS

Experimental organism

A total of 60 *Lumbriculus variegatus* (Carolina Biological Supply Company, Item #141720) worms were divided evenly among 6 sterile plastic containers (Uline, Cat. # S-17034), filled with 30 mL of distilled spring water. Containers were randomly assigned to a negative control group with un-supplemented fish food, a turmeric treatment group, or an ALA treatment group. All containers were kept at room temperature (21–23 °C) under consistent lighting conditions, with no additional aeration and monitored water levels. Lighting was kept constant to minimize any environmental

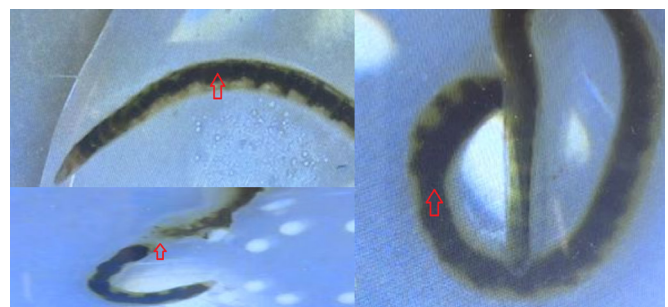


Figure 7: Non-viable *Lumbriculus variegatus* specimen excluded from analysis. The image's arrows show abnormal morphology, including altered pigmentation, tissue swelling, and reduced motility. All specimens with these characteristics were excluded from the dataset before measurements were taken.

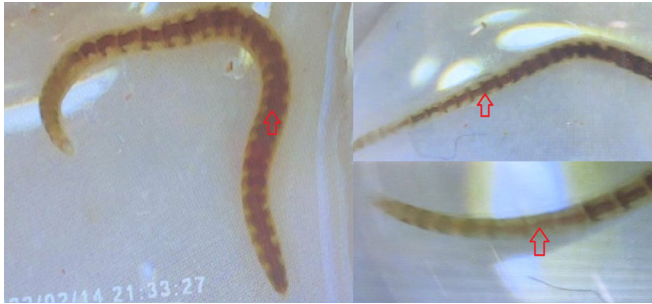


Figure 8: Viable *Lumbriculus variegatus* specimen included in analysis. The image's arrows highlight normal morphology, including uniform pigmentation, regular segment alignment, and observable pulsation. All specimens with these characteristics were included in the dataset for segment regeneration and heart rate measurements.

variability in the results, since fluctuations in light exposure have been reported to influence behavior and metabolic activity in aquatic invertebrates (17).

Amputation and regeneration monitoring

A transverse amputation approximately 2 to 3 millimeters in length was performed anterior to the clitellum using a disposable scalpel (Fisher Scientific, Cat# 22-079-707). Worms were imaged using a USB digital microscope (Elikliv, Cat# JAA103873), and regeneration was monitored for four days by counting newly formed body segments distal to the amputation site, identified by their lighter gray coloration relative to pre-existing tissue.

Supplement preparation and feeding protocol

The dietary base of the fish food flakes (Tetra, Item #16210) was supplemented with turmeric powder (Nature Made, Item #2754) or ALA powder (Nutricost) by thoroughly mixing the powdered supplement directly into dry fish food flakes to homogenize distribution before feeding. Worms were fed once daily at the same time each day throughout the experiment. The treatment concentration was maintained at 0.1 g of supplement per 10 g of food, a ratio selected to provide measurable biological exposure while avoiding overt physiological stress based on preliminary observations and prior invertebrate supplementation studies (17). Following the conclusion of data collection, residual waste was removed using 70% ethanol (Fisher Scientific, Cat# A459-500) as part of standard post-experiment sanitation to prevent microbial contamination.

Segmental Regeneration and Heart Rate Assessment

A USB Digital Microscope (Elikliv, Cat# JAA103873) was used to assess regeneration in each worm. Regeneration was quantified by counting only newly regenerated body segments distal to the amputation site on four feeding days. Newly regenerated segments were identified by their lighter coloration and uniform spacing relative to pre-existing tissue. The dorsal vessel was visualized under the microscope to measure physiological response, and heart rate was measured by counting contractions over a 30-second observation interval.

Statistical Analysis

To evaluate how turmeric and ALA performed compared to controls, one-tailed two-sample t-tests were performed using a TI-84 graphing calculator. Statistical significance was defined as $p < 0.05$.

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