Aidyn Zhuo, Kenneth Sanchez, Alexander Lee

The Effects of Trichosanthes Root Extract on Regeneration Rate, Behavior, and Movement of *Dugesia tigrina*

Abstract

This study investigated the effects of *Trichosanthes* root extract on the regeneration rate of *Dugesia tigrina*, a freshwater planarian. Planarians were chosen for their regenerative ability and stem cell systems. Traditionally used in Chinese medicine for its anti-inflammatory and antioxidant properties, *Trichosanthes* root was tested for its unexplored regenerative potential. The goal was to evaluate a cost-effective, biologically based method for enhancing tissue regeneration with potential applications in regenerative medicine and wound healing. Planarians were continuously exposed to varying concentrations of the extract, with locomotor activity recorded before and after treatments. Specimens were then transversely amputated into head and tail fragments, which were observed independently. Regenerative outcomes, including body length, eyespot formation, and indicators of nervous system recovery, were measured at regular intervals. Results demonstrated that in trial 1, group D slightly decreased in head length regeneration compared to our control group A. Although group A had the smallest amount of head regeneration over the course of 11 days, there was no significant difference found between the groups in trial 1. Similarly, group C had the least regenerative ability overall over 11 days, but there was still no statistical difference found between the groups. Ultimately, Trichosanthes root was not significantly effective in the regeneration, locomotor activity, and behavior of *Dugesia Tirgrina*. These findings suggest that even as planarians remain valuable assets in testing biological compounds, *Tricosanthes* root may not have benefited tissue regeneration as hypothesized.

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Introduction

Rationale

This study aimed to investigate whether Trichosanthes root extract impacted tissue regeneration in *Dugesia tigrina,* a planarian species known for its excellent regenerative capabilities. Although Trichosanthes root has been traditionally used in Chinese medicine for its anti-inflammatory and antioxidant properties (Luo et al., 2013), its role in promoting regeneration has remained largely unexplored. This experiment aimed to determine whether natural compounds present in the root, such as trichosanthin, flavonoids, and polysaccharides (Lo *et al.,* 2017), created a more favorable environment for tissue repair by reducing inflammation and oxidative stress (Zhang *et al.,* 2022). These conditions were known to promote cellular growth and repair, and this research provided an opportunity to explore their impact in a model organism.

Dugesia tigrina was chosen for its neoblast-based regeneration, a process similar to human stem cell activity (Rink, 2013). The study assesses whether natural herbal compounds could promote stem cell-driven repair by exposing *Dugesia tigrina* to varying concentrations of Trichosanthes root extract and observing their regeneration over time. This experiment contributed new insight into how foreign plant-based remedies might be integrated into regenerative medicine. Additionally, it offers a cost-effective and accessible way for future biomedical research, bridging traditional medicine with modern scientific understanding.

Trichosanthes Root

Trichosanthes root, derived from the plant *Trichosanthes kirilowii*, has been widely used in traditional Chinese medicine for centuries. *Trichosanthes* root extract has primarily been prescribed to treat respiratory conditions, fevers, and inflammatory diseases due to its potent

pharmacological effects (Lo et al., 2017). The root contains several bioactive compounds, most notably trichosanthin, flavonoids, triterpenoids, and polysaccharides, which are known to have antioxidant, anti-inflammatory, and immune-regulatory properties (Luo *et al.,* 2013).

Trichosanthin, a ribosome-inactivating protein, had been studied for its antitumor and antiviral effects (Luo et al., 2013). Flavonoids, another key component, act as antioxidants by scavenging free radicals and protecting cells from oxidative damage. These antioxidant effects were especially important in maintaining cellular health during stress or injury. Additionally, anti-inflammatory compounds in the root help reduce tissue swelling and immune overactivation, creating a more favorable environment for tissue growth and repair (Zhang et al., 2022). Despite its well-documented medicinal effects, minimal research has been conducted to assess *Trichosanthes* root's influence on tissue regeneration, particularly in simple model organisms. The present study addresses this gap by exploring how *Trichosanthes* compounds could impact regeneration at the cellular level.

Dugesia tigrina

Dugesia tigrina belongs to a group of flatworms known as planarians and was widely used in biological research due to its remarkable regenerative abilities. When dissected, each fragment of a planarian could regenerate the missing parts, resulting in complete and functional organisms. This extraordinary capability was made possible by the presence of neoblasts, pluripotent adult stem cells that proliferated and differentiated into various tissues (Rink, 2013).

Planarians have been used extensively to study the molecular and cellular mechanisms of regeneration, stem cell behavior, and pattern formation. Because the basic processes of cell division, differentiation, and tissue remodeling in planarians shared similarities with those in

vertebrates, they served as a valuable model for regenerative medicine. Furthermore, their low maintenance cost, rapid regeneration time, and transparent bodies made them an ideal organism for laboratory studies. In this study, *Dugesia tigrina* serves as a living model to observe whether *Trichosanthes* root extract influences the rate and quality of regeneration.

Other studies

Previous research on tissue regeneration has mostly focused on identifying chemical or genetic factors that promote cellular repair. Model organisms like *Dugesia tigrina* have been widely used because of their remarkable ability to regenerate lost body parts through neoblast activation of pluripotent adult stem cells responsible for tissue regrowth (Rink, 2013). Studies tested various compounds such as caffeine, nicotine, and turmeric to observe their effects on planarian regeneration, establishing a baseline understanding of how external substances influenced stem-cell-driven repair (Nekliudov *et al*., 2018).

However, the regenerative potential of traditional herbal medicines, particularly Chinese herbs like *Trichosanthes kirilowii* root extract, remained largely unexplored. While *Trichosanthes* had been studied extensively for its antioxidant, anti-inflammatory, and anticancer effects in human cell lines (Lo *et al.*, 2017; Zhang *et al*., 2022), its impact on in vivo tissue regeneration, especially in planarian models, had not been examined. This gap presented a unique opportunity to explore whether its bioactive compounds, such as trichosanthin, might enhance or inhibit regeneration.

This experiment was designed to fill this gap by directly observing how planarians responded to *Trichosanthes* root extract exposure during regeneration. By applying varying concentrations of the extract to amputated planarians, the study offered a novel test of traditional

herbal medicine within a regenerative biology framework. To our knowledge, this was the first study to evaluate *Trichosanthes* root extract in this context, bridging traditional herbal medicine and modern regenerative science in an accessible, cost-effective manner.

Materials and Methods

Preparation of Planarians

A total of 40 *Dugesia tigrina* planarians were obtained from Carolina Biological Supply (Catalog #132954). Specimens were housed in loosely sealed glass jars containing Poland Spring water to maintain hydration and oxygenation. The planarians were randomly distributed into four equal groups (n = 10 per group), with each group designated to receive a different concentration of *Trichosanthes* Root Extract. Jars were stored in a cabinet with adequate spacing between containers to prevent cross-contamination. During the nurturing period, planarians were fed ground beef every other day (Monday, Wednesday, and Friday).

Planarian Maintenance and Treatment

Before experimentation, all planarians underwent a one-week nurturing period. Throughout this time, standard laboratory safety protocols were followed. Experimental materials and surfaces were sterilized using a 70% isopropanol solution. Feedings were conducted up to three times per week. Following each feeding, the meat and planarian were removed from the jars separately using pipettes and tweezers for the meat. Then the jars were cleaned and filled with Poland Spring water. Pipettes were then used to transfer the planarian back into the jars. This maintenance regimen was sustained for seven consecutive days before initiating experimental procedures.

Table 1. The concentration of *Trichosanthes* Root Extract and Water Solution.

Group	Concentration of *Trichosanthes* extract	Amount of *Trichosanthes* Extract (µL)	Amount of Poland Spring Water (mL)	Number of Organisms	Duration of treatment (Days)
A	0%	0	50	10	7
B	.01%	5	50	10	7
C	.03%	15	50	10	7
D	.05%	25	50	10	7

Preparation of Treatment Solutions

All preparation procedures were conducted under sterile conditions. Work surfaces were disinfected using a 70% Isopropanol solution, and hands were washed with soap and water before beginning. Standard personal protective equipment (PPE), including laboratory coats, gloves, and safety goggles, was worn throughout the process. The *trichosanthes* extract was purchased on Amazon (HerbalTerra). Materials required for treatment preparation included beakers, graduated cylinders, pipettes, an analytical balance, weighing boats, *trichosanthes* extract, and Poland Spring water. The volume of Poland Spring water specified in Table 1 was measured using a graduated cylinder and poured into a beaker. The appropriate volume of

Trichosanthes root extract, also specified in Table 1, was measured by the micro pipette and was pipetted into the beaker. The solution was mixed by gently swirling the beaker. This procedure was repeated for each treatment group (A–D), based on the concentrations indicated in Table 1. Upon completion, all materials were cleaned, disinfected, and returned to their designated storage locations.

Treating Planarians

To start the experiment, all planarians underwent a one-week nurturing period. Throughout this time, standard laboratory safety protocols were followed, including the use of PPE. Experimental materials and surfaces were sterilized using 70% isopropanol before each session. Each group was soaked corresponding to the treatment conditions detailed in Table 1 for around 24 hours.

Mobility and Behavioral Assessment

Baseline locomotor activity was assessed before treatment administration. All preparatory procedures were conducted under sterile conditions, with surfaces, tools, and PPE disinfected using 70% isopropanol. For the assay, 30 mL of Poland Spring water was added to each of four sterile petri dishes. Planarians were transferred into their respective dishes according to group assignment. Graph paper was placed beneath each petri dish as a spatial reference (see Figure 1), and a video recording was taken for 30 seconds to document the number of tiles crossed and behaviors such as c-like shape.

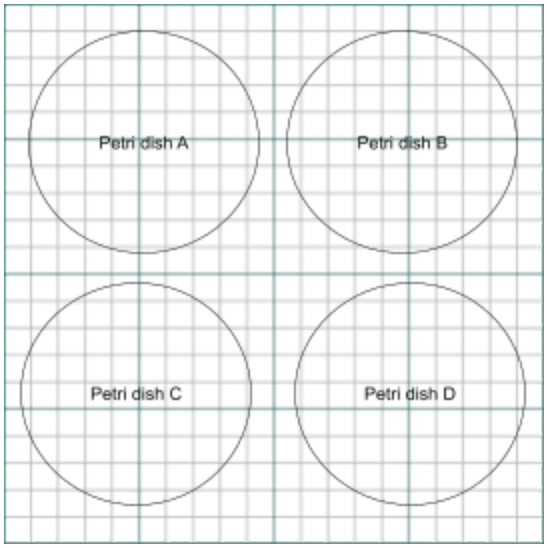

Figure 1. Experimental setup for behaviors and movement.

Planarian Dissection

Before dissection, the scalpel was sterilized using a paper towel moistened with 70% isopropanol. Group A planarians were immobilized by placement on ice for one minute to minimize movement. Each specimen was individually dissected before the pharynx using the sterilized scalpel. To prevent cross-contamination, the scalpel was re-sterilized after every three dissections. The same procedure was subsequently applied to Groups B, C, and D. Following dissection, tissue fragments were sorted into labeled containers: head fragments from Group A were placed into a jar labeled “AH” (A Heads), while tail fragments were placed into a jar labeled “AT” (A Tails). This labeling system was consistently applied to the other experimental groups, resulting in containers BH, CH, DH (head fragments) and BT, CT, DT (tail fragments).

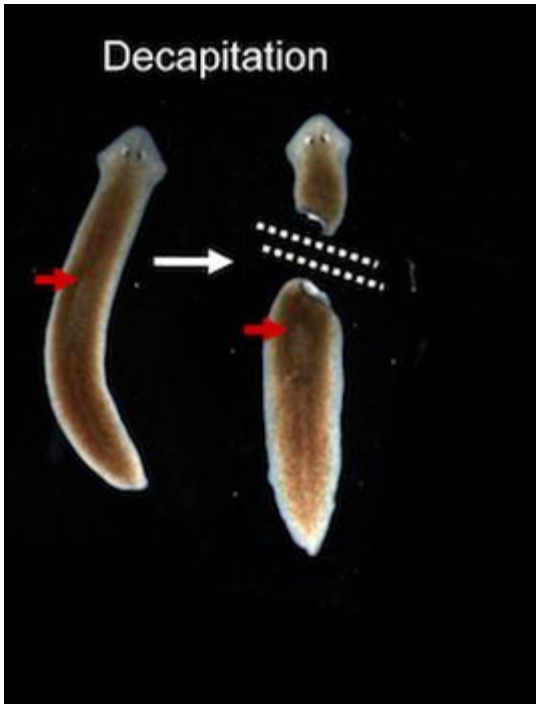

Figure 2. How to prepare a planarian for tissue regeneration

https://images.app.goo.gl/8wutdNqguqyf2f4LA

Length Regeneration Assay

To assay length regeneration, standard lab safety protocols were followed, including wearing safety goggles and aprons. All materials and workspaces were disinfected before beginning the experiment. Body lengths of all planarians were measured in millimeters using a ruler. This process was repeated every other business day. Once testing concluded each day, planarians were returned to their cleaned containers, and all workspaces were disinfected.

Eyespot Regeneration Assay

To assess eyespot regeneration, standard lab safety protocols were followed, including wearing safety goggles and aprons. All materials and workspaces were disinfected before beginning the experiment. Planarians were not fed until their pharynx had fully regenerated to

avoid any interference with regeneration. Specimens from the treatment group ("T") were transferred to petri dishes and placed under a light microscope. Each planarian's tail region was photographed and observed daily for two weeks. Regeneration progress was scored using a standardized scale: 0 for no regeneration, 0.5 for partial regeneration, and 1 for full regeneration.

Touch Regeneration Assay

Touch Responsive was measured following the same safety and preparation procedure. After ensuring that the pharynxes of the planarians had fully regenerated, specimens from the "T" group were gently transferred to petri dishes using pipettes. A soft-bristle brush was used to lightly touch each planarian. Reactions were observed and recorded to determine the responsiveness. This procedure was repeated daily on business days until consistent responses were observed. Once testing concluded each day, planarians were returned to their cleaned containers, and all workspaces were disinfected.

Post Experiment

At the conclusion of the study, all specimens were ethically euthanized by autoclaving at 121°C for 20 minutes under supervisor oversight.

Data analysis

All data were recorded and stored electronically in Microsoft Excel for subsequent analysis. Planarian regeneration was assessed by comparing eyespot regeneration scores over time between tail-amputated groups, visualized using multiple line graphs. Body length measurements were the head to and tail regions using a ruler. Regeneration in overall body length over time was analyzed through additional line graphs comparing tail and head groups.

Planarian movement was quantified by counting the number of lines each planarian crossed over time, and the results were displayed using bar graphs. Reactions to the touch test were measured by observing the responsiveness of each planarian when stimulated with a soft-bristle brush; changes in response over time were represented in line graphs to compare between treatment groups. Behavioral changes were assessed by counting the number of C-like body shapes formed over time and comparing frequencies between groups using bar graphs. Mean and standard deviation were calculated using standard Excel functions. Statistical significance of the data was determined using ANOVA (Analysis of Variance), followed by Tukey's HSD post hoc test via the platform at https://www.socscistatistics.com/tests/anova/default2.aspx. P-values less than 0.05 were considered statistically significant.

Results

The movement, behavioral, and regenerative assays were conducted to evaluate the effects of *trichosanthes* root extract on planarians. The behaviors, regenerative, and movement measures indicate minimal effects from the *trichosanthes* root extract on planarians. There were a few statistically significant results, but they did not form a clear trend.

Mean movement activity, quantified as lines crossed per day, did not differ significantly among the 0%, 0.01%, or 0.03% extract concentrations in either trial ($p > 0.05$). All groups' movements were compared over time, indicating that exposure to *Trichosanthes* root extract did not alter baseline locomotor function (Figures 3 and 9).

The c-shape curling frequency was measured, and extract-induced planarian's c-shape curling frequency remained consistent across all concentrations, and statistical analysis revealed no significant treatment effects. ($p > 0.05$; Figures 4 and 10).

Eyespot regeneration proceeded normally in all groups, with increases in eyespot score observed over time. Although qualitative trends suggested a slight enhancement in extract-treated individuals, no statistically significant differences among treatment concentrations were detected in either trial ($p > 0.05$; Figures 5 and 11).

Head tissue regeneration exhibited isolated significant differences among groups. In Trial 1, extract concentration significantly affected regeneration length on Day 1 ($p < 0.001$) and Day 5 ($p = 0.002$), but no consistent dose-dependent pattern was observed thereafter (Figure 6). Similarly, in Trial 2, significant variation occurred only on Day 1 ($p < 0.001$), with subsequent days showing no significant differences among treatments (Figure 12). Collectively, these findings suggest that any early-stage differences were transient and not indicative of sustained extract-mediated modulation of head regeneration.

Tail regeneration length demonstrated significant differences across treatment groups on multiple time points, including Day 1 ($p < 0.01$), Day 3 ($p < 0.0001$), Day 5 ($p < 0.02$), Day 10 ($p < 0.0001$), and Day 11 ($p < 0.01$). Despite these statistical differences, regeneration trajectories did not exhibit clear dose-response relationships, suggesting that the observed effects were limited and not biologically robust (Figure 7).

Touch responsiveness was uniformly high across all treatment groups, with all planarians responding to tactile stimuli throughout the experiment. No significant differences were detected at any time point ($p > 0.05$; Figure 8).

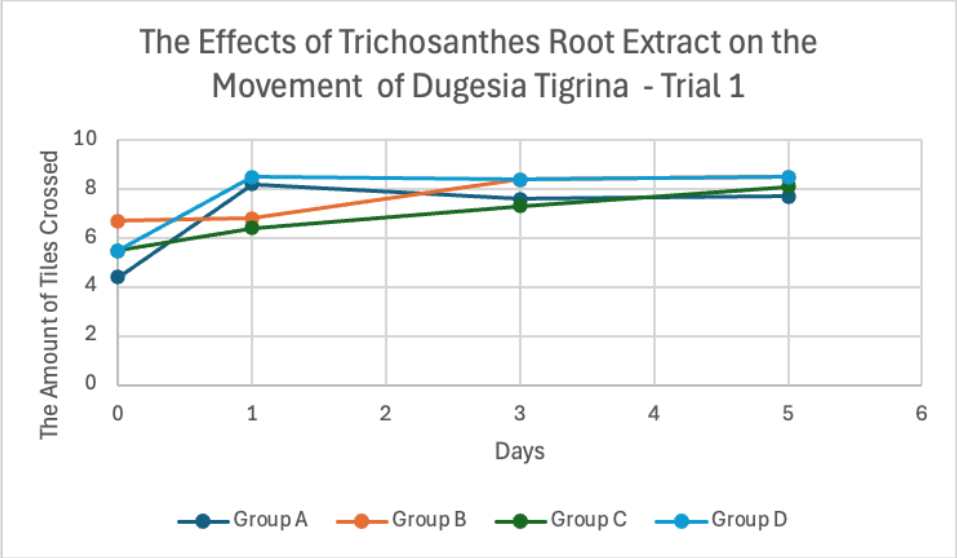

Figure 3. The Effect of *Trichosanthes* Root Extract on *Dugesia tigirina* Movement-Trial 1. Each line represents the mean lines crossed per day of planarian (n=10 per group), different concentrations of *trichosanthes* root extract (A: 0% of *Trichosanthes* Root Extract, B: 0.01% of *Trichosanthes* Root Extract, 0.03% of *Trichosanthes* Root Extract, and D: 0.01% of *Trichosanthes* Root Extract). The results indicate that there are minimal effects of *trichosanthes* root extract on the planarian movement. No significant differences were observed between groups at any time point ($p > 0.05$)

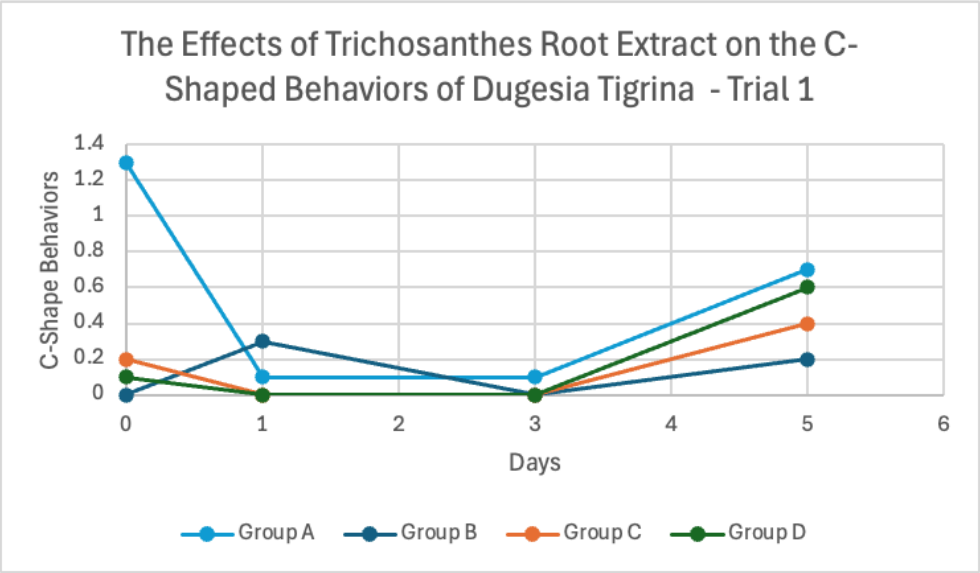

Figure 4. The Effects of *Trichosanthes* Root Extract on the C-Shape *Dugesia tigrina* - Trial 1. Each line represents the c-shape curl behavior of planarian (n=10 per group) at different concentrations of *trichosanthes* root extract. The results indicate that *trichosanthes* root extract had minimal effects. No significant differences were observed between groups at any time point ($p > 0.05$).

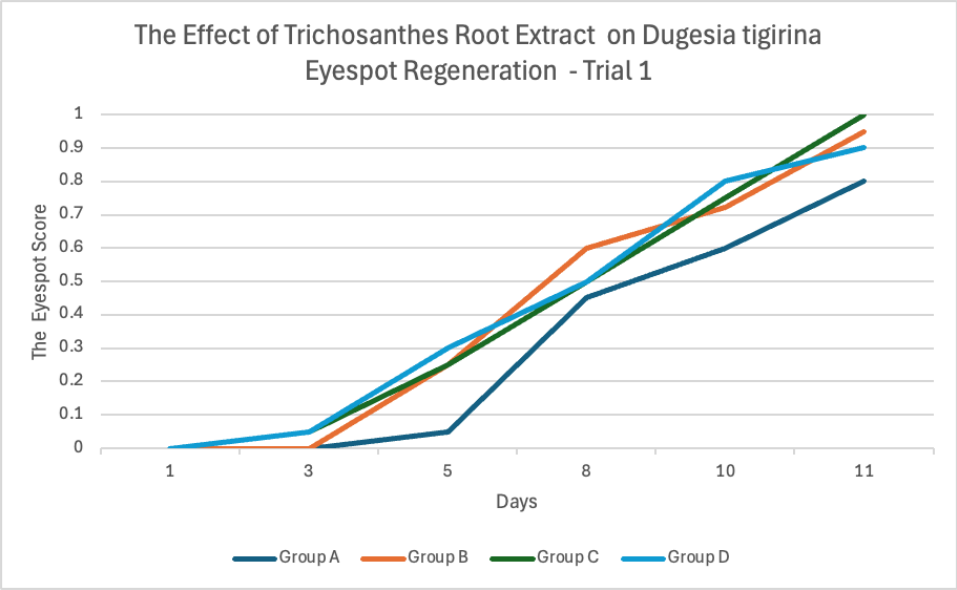

Figure 5. The Effects of *Trichosanthes* Root Extract on *Dugesia tigrina* Eyespot Regeneration - Trial 1. Each line represents the mean eyespot score of the planarian (n=10 per group) at different concentrations of *trichosanthes* root extract. The results indicate that there are positive effects of *trichosanthes* root extract on the eyespot regeneration. No significant differences were observed between groups at any time point ($p > 0.05$)

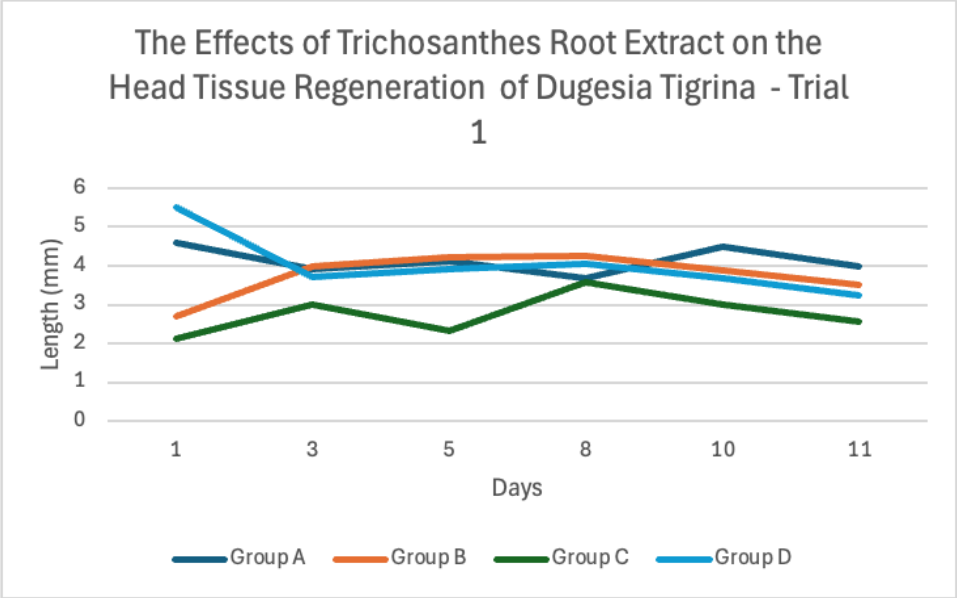

Figure 6. The Effects of *Trichosanthes* Root Extract on the Head Tissue Regeneration of *Dugeisa Tigirina* - Trial 1. Each line represents the amputated planarian (n=10 per group) mean head regeneration length of different concentrations of *trichosanthes* root extract. Significant effects were observed on Day 1 ($p < 0.001$) and Day 5 ($p = 0.002$), using ANOVA with Tukey's HSD, but *trichosanthes* root extract demonstrated minimal overall impact on planarian head regeneration.

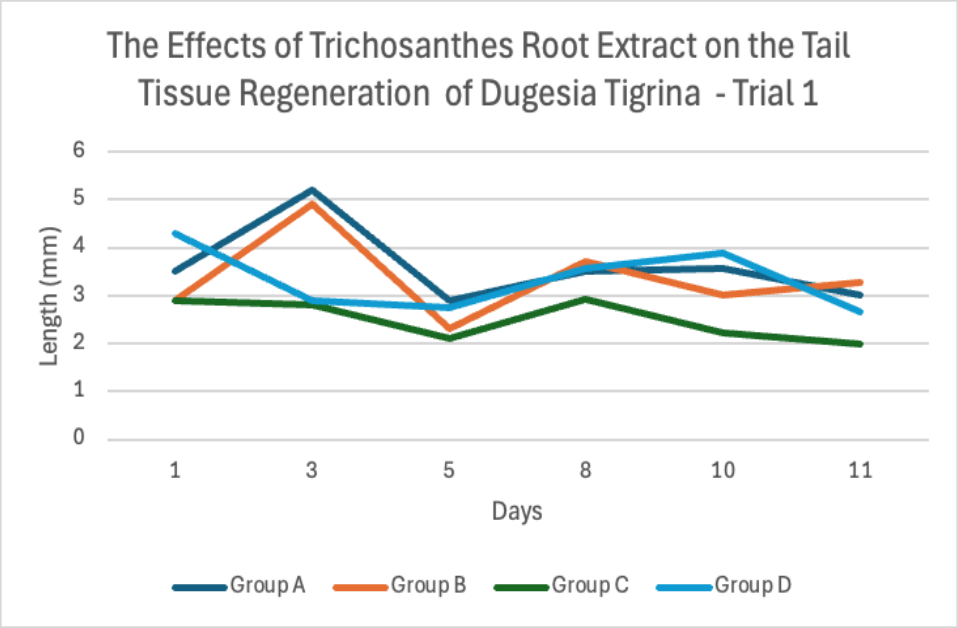

Figure 7. The Effects of *Trichosanthes* Root Extract on the Tail Tissue Regeneration of *Dugesia TTigirina- Trial 1.* Each line represents the amputated planarian (n=10 per group) mean tail regeneration length of different concentrations of *trichosanthes* root extract. Significant effects were observed on Day 1 ($p < 0.01$), Day 3 ($p < 0.0001$), Day 5 ($p < 0.02$), Day 10 ($p < 0.0001$), and Day 11 ($p < 0.01$) using ANOVA with Tukey's HSD, but *trichosanthes* root extract demonstrated minimal overall impact on planarian's tail regeneration.

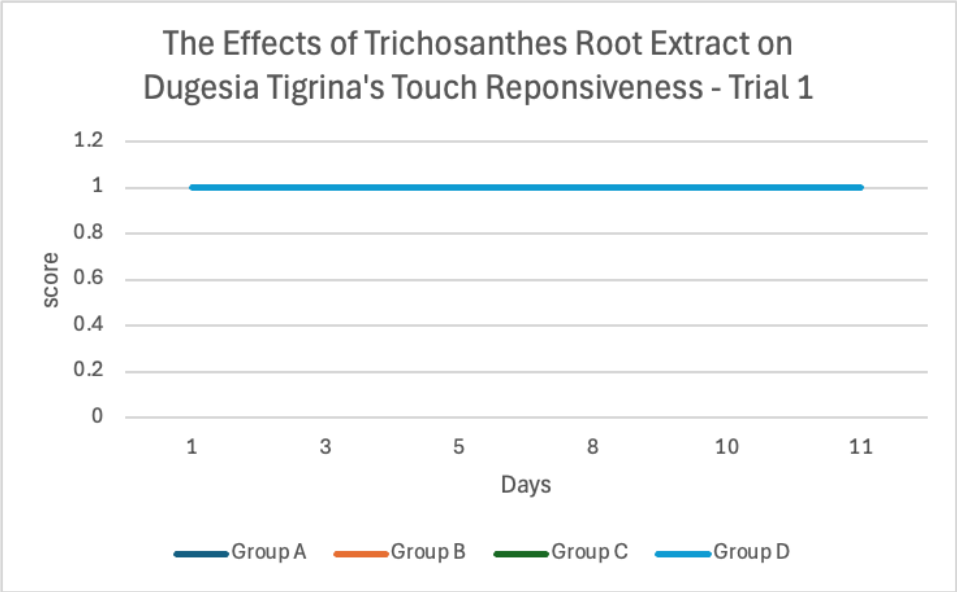

Figure 8. The Effects of *Trichosanthes* Root Extract on Touch Responsiveness on *Dugesia tigrina's* Touch Responsiveness - Trial 1. Each line represents the planarian (n=10 each group) mean touch-responsive score of different concentrations of *trichosanthes* root extract. The findings indicate that all the planarians had responded when touched. No significant differences were observed between groups at any time point ($p > 0.05$)

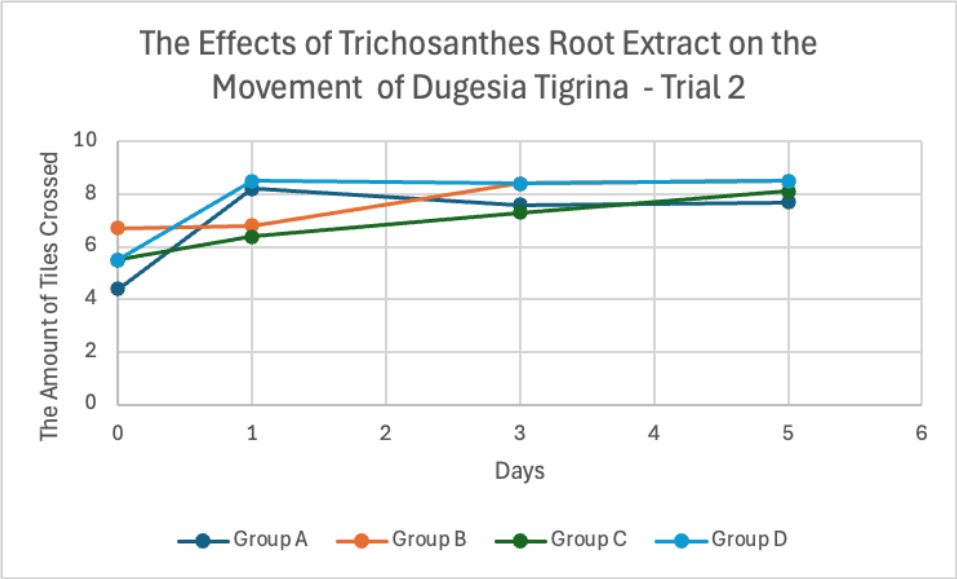

Figure 9. The Effect of *Trichosanthes* Root Extract on the Movement of Dugesia Tigirna - Trial 2. Each line represents the mean lines crossed per day of planarian (n=10 per group) at different concentrations of *trichosanthes* root extract. The data indicate that *trichosanthes* root extract has minimal effects on the movement of planarians. No significant differences were observed between groups at any time point ($p > 0.05$)

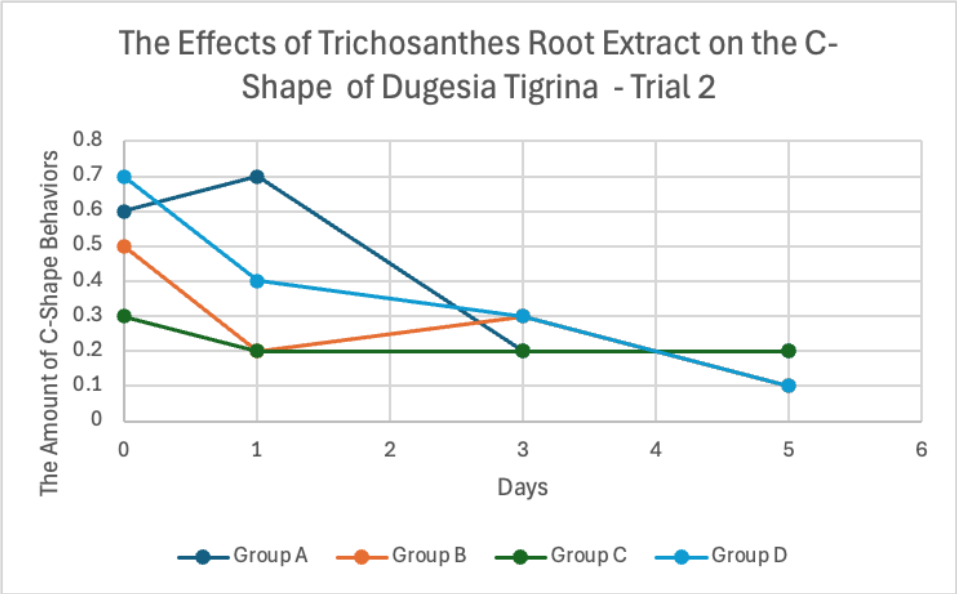

Figure 10. The Effects Of *Trichosanthes* Root Extract on the C-Shape behaviors of *Dugesia tigrina*. Each line represents the c-shape curl behavior of planarian (n=10 per group) different concentrations of *trichosanthes* root extract. The results indicate that *trichosanthes* root extract had minimal effects. No significant differences were observed between groups at any time point ($p > 0.05$)

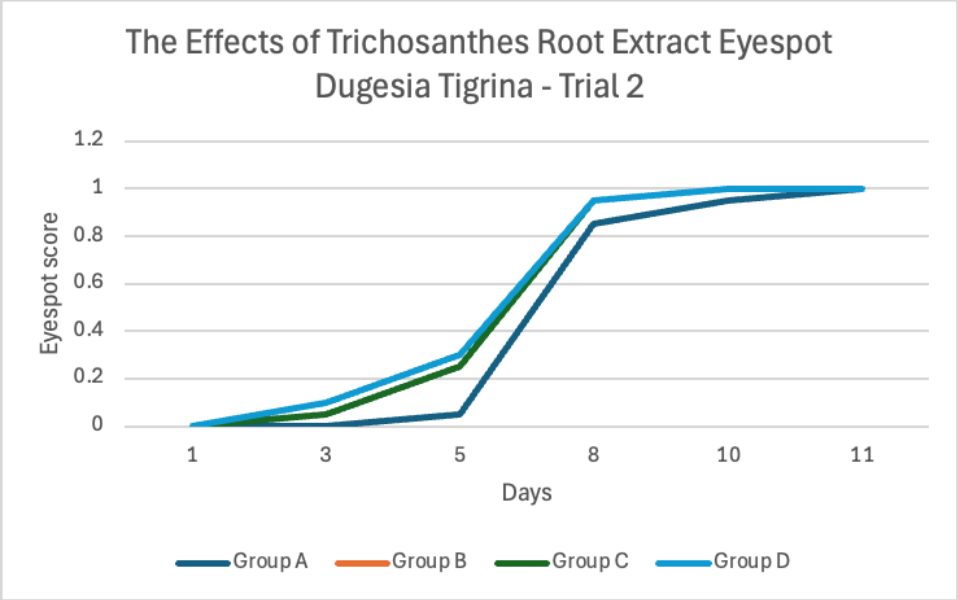

Figure 11. The Effects of *Trichosanthes* Root Extract on *Dugesia tigrina* Eyespot Regeneration - Trial 2. Each line represents the mean eyespot score of the planarian (n=10 per group) at different concentrations of *trichosanthes* root extract. The results indicate that there are positive effects of *trichosanthes* root extract on the eyespot regeneration. No significant differences were observed between groups at any time point ($p > 0.05$).

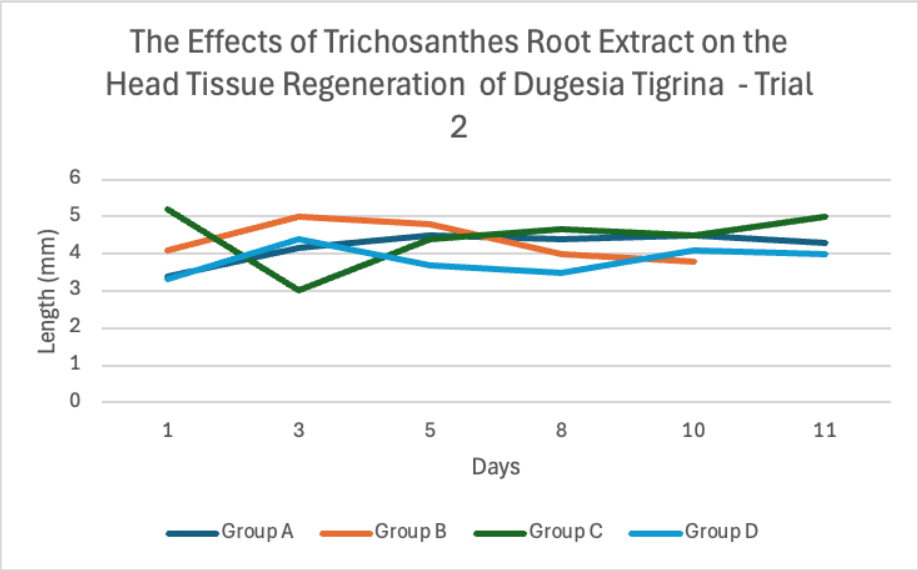

Figure 12. The Effects of *Trichosanthes* Root Extract on the Head Tissue Regeneration of *Dugeisa Tigirina* - Trial 2. Each line represents the amputated planarian (n=10 per group) mean head regeneration length of different concentrations of *trichosanthes* root extract. Significant effects were observed on Day 1 ($p < 0.001$) and Day 11 ($p > 0.05$), using ANOVA with Tukey's HSD, but *trichosanthes* root extract demonstrated minimal overall impact on planarians head regeneration.

Discussion and Conclusions

The hypothesis predicted that *Trichosanthes* root extract would benefit regeneration rate, behavior, and mobility in *Dugesia tigrina*. However, the results did not support this prediction. Across both trials, the extract did not produce significant differences in eyespot recovery, c-shape behavior, or movement compared to the control group. Although some groups showed small trends, such as slight decreases in head regeneration in Group D or lower overall regeneration in Group C. These findings show that *Trichosanthes* root extract did not enhance regeneration in planarians due to these differences being minimal and inconsistent. This helped narrow down which natural foreign substances may be useful for future regenerative medicinal studies.

Numerous problems arose during the experiment that could have affected the data. Forms of contamination occurred between a few groups when misusing pipettes for the incorrect groups, which introduced various concentrations of *Trichosanthes* root extract to the planarian. Uneven cuts during dissection caused variations in the size and shape of head and tail fragments, leading to differences in the recovery of the tissue. There were many days when data could not be collected, which created gaps in the schedule and made some trends harder to track accurately. In addition, multiple jars of planarians were left dirty and unmaintained, which may have affected water quality, stress levels, and the general health of the organisms. These issues could be improved by stricter sterilization between groups, more precise cutting tools, a more stable data-collection schedule, and more frequent and proper cleaning of the jars.

Future research could utilize differing concentrations of the root extract, as well as potentially different forms of treatment. With the rise of AI, its tools can be utilized to analyze data, which would provide more time for discussions, as well as eliminate human error. Although testing *Trichosanthes* root extract expanded our knowledge on Chinese herbal medicines, the testing of varying branches of Chinese herbal medicine introduces new compounds that will likely provide new data.

In conclusion, the data from both trials supported the hypothesis, but were negligible in terms of regeneration, behavior, and movement in the planarian. Although there were various gaps and limitations to our work, this experiment still proved to be beneficial, as although our data was negligible, an understanding of Chinese herbal medicines was gained and expanded on.

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