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Enhancement of wound healing in fruit fly larvae with poppy seeds and turmeric

Singh A

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Abstract

Turmeric (*Curcuma longa*) and poppy seeds (*Papaver somniferum*) are two herbs that have been investigated for their nutritional, antioxidant, and anti-inflammatory properties. However, these compounds have seldom been tested as orally administered wound healing agents. These common compounds could possibly be used as inexpensive and accessible wound treatments. Using *Drosophila melanogaster* as a model organism, this study analyzed the effects of *C. longa* and *P. somniferum* diets on decreases in wound size. *D. melanogaster* flies were cultured for two weeks. Four treatment media were prepared: a negative control without additives, one containing *P. somniferum*, one containing *C. longa*, and one containing *Aloe vera* as a positive control—studies have shown that *Aloe vera* works as a wound healing agent when ingested orally or applied topically. 15 flies (9 female virgins, 6 males) from the initial culture were then placed in each of the four treatment groups, and after two weeks, 20 third-instar larvae were removed from each group and wounded. The larvae were monitored over the course of 8 hours, and percent changes in wound size were recorded and analyzed using ImageJ software. Average percent decreases in wound size from hour 2 to hour 8 after wounding were as follows: -35.3%, -41.2%, -50.5% and -45.0% for the control, *P.somniferum*, *C.longa* and the *Aloe vera* groups respectively. All three treatments significantly shortened the wound healing time in *D. melanogaster* larvae. These results corroborate similar other findings in making the case for using these compounds as low-cost, accessible, and effective herbal oral supplements or drugs to better treat wound injuries in developing regions.

Keywords

Papaver somniferum, *Curcuma longa*, Herbal treatments, Wound healing, Herbal wound healing, *Drosophila melanogaster*, Oral medications for wounds, Poppy seeds, Turmeric powder, Fruit fly larvae, *Aloe vera* powder, Herbs

Anusha Singh, Vestavia Hills high school, 2235 Lime Rock Rd., Vestavia Hills, AL 35216, USA, anushasinghswa@gmail.com

Introduction

There are two main types of wounds: acute and chronic. Acute wounds occur suddenly and heal in a reasonable and predictable time (1). Chronic wounds do not proceed through the normal stages of healing in a timely manner. They may arise from complications of an acute wound or due to underlying health conditions (2).

The goal of this study was to determine if the time taken for the wound healing process could be shortened, thereby lowering the risk of complications such as infection, which cause the progression of an acute wound to a chronic wound. Many lab-developed topical treatments for acute wounds have been discovered and explored, and access to these topical treatments has increased in recent years. However, certain herbal treatments could be lifesaving for people who may not be able to afford the wait or cost of lab-developed medicines.

If commonly found and cultivable herbs such as poppy seeds or turmeric are found to modulate the immune system and benefit the wound-healing process, these herbs could have a significant effect on illness and mortality rates in developing regions of the world. This is because in developing countries, wounds account for a much greater proportion of disease and mortality when compared to developed countries. Over 90% of injury-related deaths occur in low- and middle-income countries (3). Consequently, this study researched the effects of *Curcuma longa* powder and *Papaver somniferum* seeds on wound healing in *Drosophila melanogaster*.

Curcuma longa, also known as turmeric, is a plant whose rhizomes are widely used in Indian and Middle Eastern cuisines. Its chemical properties have promising implications in medicine (4). *C. longa* has shown anti-inflammatory, antioxidant, and antimicrobial properties in several studies. *C. longa* has been beneficial as a topical wound-healing agent, as shown in many studies. However, studies of its wound healing properties when ingested as an oral medication or as part of a long-term diet are insufficient (5).

Poppy seeds, oilseeds obtained from the *Papaver somniferum* (opium poppy) plant, are commonly used in baking and in preparing tea. These seeds contain trace amounts of opium compounds. Poppy seeds are claimed to have a wide range of health benefits, because they contain high amounts of protein, fiber, and polyunsaturated fats (6). *Aloe vera* has been tested as a wound healing agent in several previous studies and shown to enhance wound healing when applied topically or ingested orally (7, 8). It is also an herbal treatment, like *C. longa* and *P. somniferum*. Therefore, it was used as a positive control for this study.

D. melanogaster was used as a model organism, as their genome is 60% homologous to that of humans, less redundant, and has homologs for about 75% of the genes responsible for human diseases (9). *D. melanogaster* is a valuable model to study epidermal wound healing because of the similarities of the wound healing processes between this invertebrate and vertebrates (10).

Both *P. somniferum* and *C. longa* are potential low-cost remedies of wound healing and are widely available in South and East Asian countries. By testing the effects of these compounds taken orally on wound healing in *D. melanogaster*, using *A. vera* as a positive control, these compounds may be further investigated as orally active wound healing agents.

Materials

The materials used were Flightless Fruit Flies - *D. melanogaster* (Imagitarium 973033), Formula 4-24® Instant Drosophila Medium (Carolina Biological 173200), Aloe Vera Leaf Powder (Mountain Rose Herbs), Turmeric Root Powder (Mountain Rose Herbs), and Poppy Seeds (Great Value/Walmart 876731438, 2780 John Hawkins Pkwy, Hoover, AL 35244, USA). All materials except for the poppy seeds were obtained online.

Methods

The food medium for the *D. melanogaster* flies was prepared for use by hydrating the dry medium with a 1:1 ratio of water to medium. Since the medium was in the form of a flaky powder, its volume could be measured in mL. 4 mL of the hydrated medium was added to each of two 15-mL vials. The remaining dry medium was stored in a dry area at 23°C for future use.

D. melanogaster flies were cultured in the two vials containing the food media. The vials were plugged with hydrophobic cotton to avoid condensation that could interfere with air supply. The flies were cultured for two weeks, which was enough time to ensure the flies reproduced to an adequate amount.

The medium for the four treatment groups was prepared as follows: first, dry medium for the three treatment groups was mixed, the *P. somniferum* group's medium was prepared by adding 1 mL of ground poppy seeds to 8 mL of dry medium, the *C. longa* group's was prepared by adding 1 mL of turmeric powder to 8 mL of dry medium, and the *A. vera* group's was prepared in the same manner, by adding 1 mL of *A. vera* powder to 8 mL of dry medium. This concentration of 11% was chosen to be potent enough that the herbs' active ingredients would take effect, without overpowering the dosage. This concentration was kept constant across all three treatment groups. 4 mL of each dry treatment medium, as well as 4 mL of regular dry medium, were each hydrated with 4 mL water and added to their respective 15 mL vials. The remaining dry media from each treatment group were stored in a dry area at 23°C.

After the preparation of the four media, the *D. melanogaster* culturing vials were placed in a freezer for five minutes at -18°C, to render them motionless for their transfer to the four vials.

With the aid of an ice pack to keep the flies motionless, 15 flies (9 female virgins, 6 males) were transferred from the culturing vials to each of the four treatment groups. Once again, these flies were cultured for two weeks. By the end of this culturing period, each group of flies had produced larvae that fed solely on their respective treatment mediums.

The flies were wounded using the following procedure. The four treatment vials were placed in a freezer for five minutes at -18°C, to render them motionless for the removal of larvae. The remaining dry media from each treatment group were hydrated and prepared. The hydrated media were filled into 20 wells in each of four separate well plates, with each plate corresponding to one of the four treatment groups. Then, 20 third-instar larvae were removed from each of the four treatment vials and punctured using a 0.2 mm steel needle at their dorsal midline.

D. melanogaster third-instar larvae were chosen to be wounded for two main reasons. First, *D. melanogaster* flies feed most as third-instar larvae, allowing us to focus our analysis on the oral benefits of the treatments (11). Second, they are easiest to work with, because as larvae they are less mobile than eclosed adult flies, and as third-instar larvae they have the largest surface area out of all larval stages. These factors reduced variation between the fly wounds and minimized errors (12). Immediately afterwards, each larva was viewed under a microscope at 100x magnification, and images of each wound were captured through the lens using a camera. After viewing, each larva was placed into its own well in the prepared well plates, corresponding to its respective treatment group. This wound-viewing process was repeated at 2, 4, 6, and 8 hours after wounding, and images of each larva's wounds were captured.

The images of each larva's wound healing timeline were uploaded to ImageJ (https://ij.imjoy.io/), an open-source software developed by the National Institutes of Health. Using the Freehand Selections option, the outline of each wound was traced, then the area of each wound was calculated by navigating under Analyze > Measure. The area of each wound was measured in image pixels, which were consistent throughout each image (3042x4032), allowing comparison of wound size across images. Figure 1 shows the use of ImageJ to calculate a wound's size.

The percent differences in wound area size for the larvae in each of the four treatment groups were calculated. The percent change in wound size from Hour 2 to Hour 8 of each larva was used to determine whether each of the herbs influenced wound healing. The significance threshold for statistical comparison between each of the three treatment groups and the control group was set at 0.0167 (Bonferroni correction; 0.05 divided by 3 to account for 3 treatment groups).

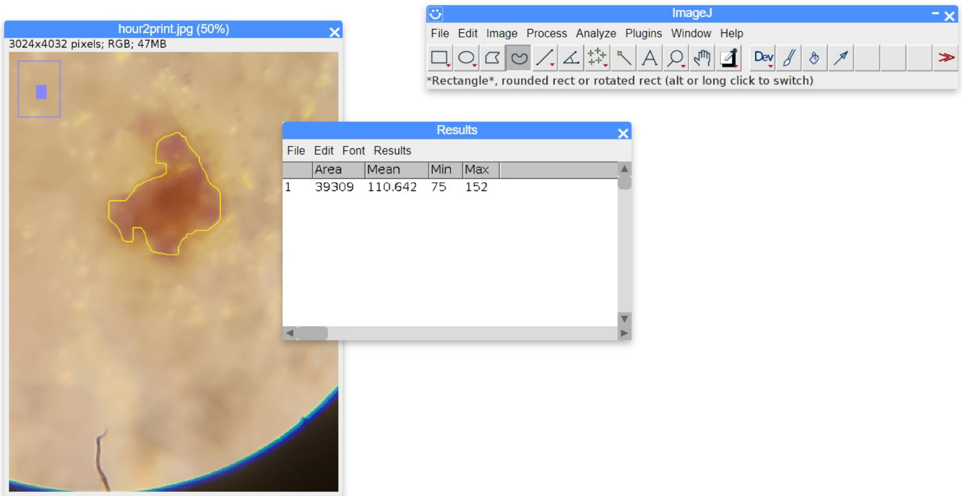

Figure 1. The use of ImageJ to find the areas of fly wounds. ImageJ was opened at https://ij.imjoy.io/. File > Open was used to upload an image. The Freehand Selections tool was selected to trace the wound's outline. Analyze > Measure was used to measure the area of the traced region, which was shown in pixels under "Area."

Results

Pairwise t-tests between each of the three treatment groups and the control group showed that there were statistically significant decreases in wound size for all three treatments.

Average percent decrease in wound size from Hour 2 to Hour 8 was the greatest in the *C. longa* treatment group (-50.485% ± 1.763%), followed by the *A. vera* group (-44.987% ± 2.626%), in turn, followed by the *P. somniferum* group (-41.234% ± 3.567%), and finally the control group (-35.332% ± 1.857%); shown are 95% confidence intervals. The p-values from the t-tests between each treatment group versus the control group were 0.0101, <0.0001, and 0.0023 for *P. somniferum*, *C. longa* and *A.vera* respectively. Each p-value was less than the 0.0167 significance threshold established prior to experimentation. Therefore, the differences in average wound size between the treatment groups and the control were considered to be significant. Figure 2 shows a graphical representation of this data.

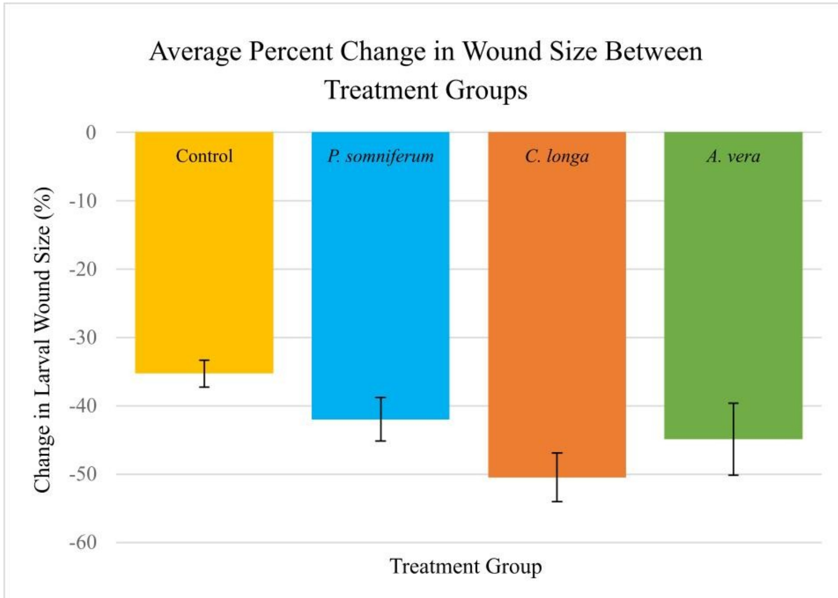

Figure 2. Average percent change in wound size between treatment groups The average percent decrease in wound size was largest in the *C. longa* treatment group (-50.485%), then the *A. vera* group (-44.987%), then the *P. somniferum* group (-41.234%), and finally the control group (-35.332%). Standard error margins (±2SE) have been accounted for as well. As shown, the error bars of all three treatment groups do not overlap with the control group's error bars.

Table 1. Larvae mortality across treatment groups

Group	Percent Mortality	Z value	P value
Control	30%	NA	NA
P. somniferum	15%	1.14	0.26
C. longa	20%	0.73	0.47
A. vera	25%	0.35	0.72

While most larvae from each group survived, mortality was observed in all four groups. Table 1 shows an overview of mortality data. Each group consisted of 20 larvae. Six larvae in the control group (30%), three larvae in the *P. somniferum* group (15%), four larvae in the *C. longa* group (20%), and five larvae in the *A. vera* group (25%) did not survive to Hour 8. Figure 3 shows which larvae did not survive and when they were found to be dead.

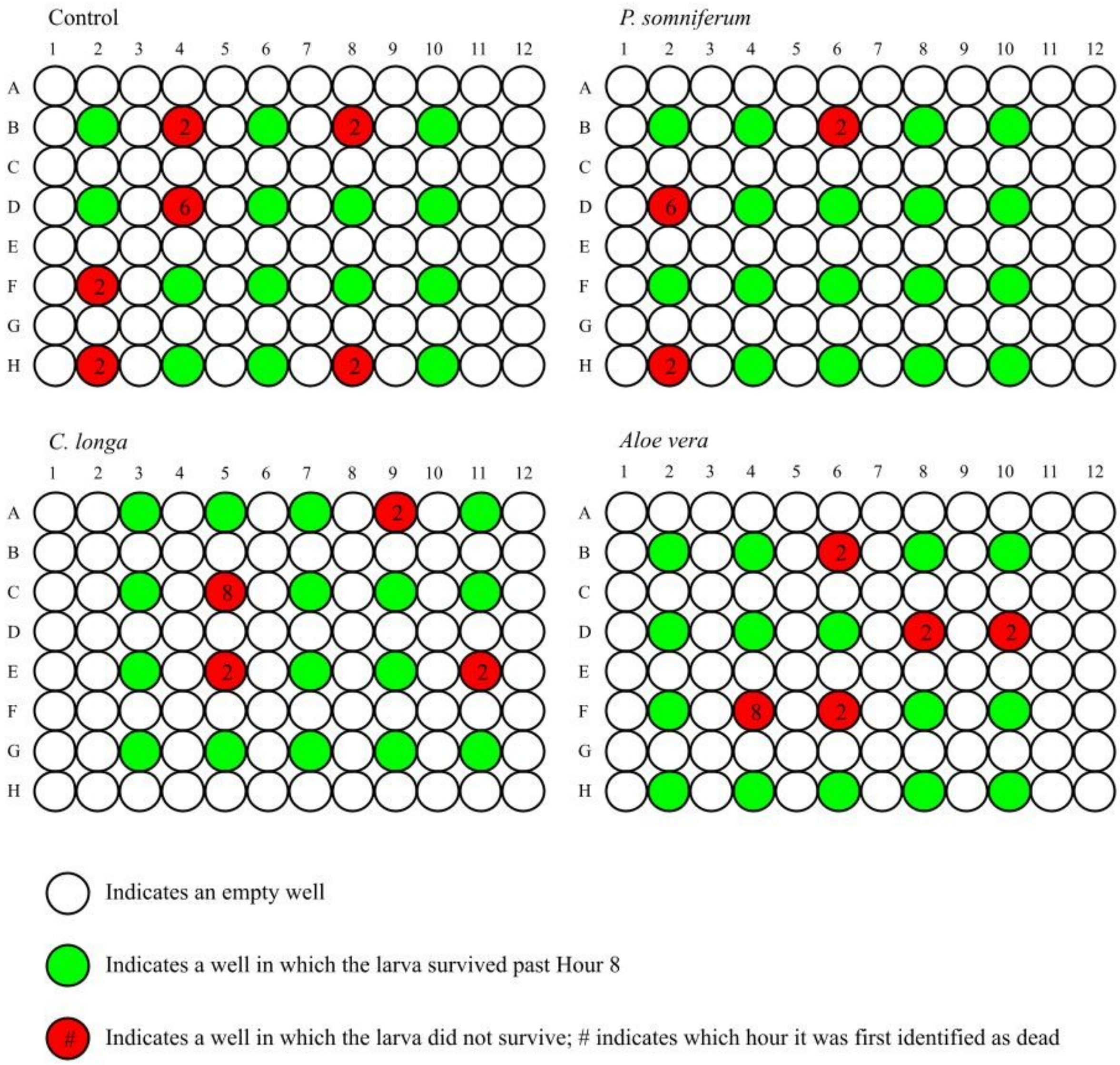

Figure 3. Graphical representation of mortality in each well plate. This graphical representation of the four well plates shows which larvae did not survive, and when they were found to be dead.

While the goal of this study was to determine whether the tested herbs helped accelerate the larval wound healing process, these varying mortality levels between groups prompted a test to determine whether the herbs tested also had a significant effect on mortality.

To determine whether these differences in mortality were significant, a z-test was performed comparing the proportions of mortality between each treatment group and the control group. As shown in Table 1, each p-value was greater than the 0.0167 significance threshold established prior to experimentation. Therefore, the differences in mortality between treatment groups were not significant.

Discussion

The purpose of this research study was to determine whether fruit flies fed with diets of *P. somniferum* or *C. longa* demonstrated larger percent decreases in wound size in *D. melanogaster* between 2 and 8 hours after injury. *A. vera* was used as a positive control to validate the research procedure. The data found shows that diets that included *P. somniferum*, *C. longa*, and *A. vera* all resulted in significantly larger decreases in wound size than the control group.

The flies treated with *C. longa* demonstrated the largest average percent decrease in wound size, that was greater than that of the positive control group, although the difference between the two was not statistically significant. The p-value between the control group and the *C. longa* group was < 0.0001, demonstrating a very significant effect. Previous research, although limited, has shown that *C. longa* may affect several mechanisms of the wound-healing process in humans by enhancing granulation tissue formation, collagen deposition, tissue remodeling and wound contraction when used topically (13). The wound healing process in mammals is similar to that of *D. melanogaster*, with common characteristics such as inflammation and cell migration (10). This provides a possible explanation for why *C. longa* had such a significant effect on wound healing in *D. melanogaster*.

The positive control group, *A. vera*, demonstrated, on average, the second-largest decrease in wound size. A significant decrease in wound size was expected, as prior research has shown that both topical and oral applications of *A. vera* benefit several aspects of health, including wound healing (7).

P. somniferum demonstrated the lowest average percent decrease in wound size out of the three treatment groups. Research on the oral or topical applications of poppy seeds on wound healing is extremely limited, but previous studies have highlighted the nutritional benefits of *P. somniferum*. A common point of concern with poppy seeds is the opium content found in the latex of the seed. However, the concentration of opium found in these seeds is extremely low and has no identifiable effects unless consumed in abnormally large amounts (14). Poppy seeds are legally available for use, and often used in the culinary arts.

While the original plan for calculating percent decreases in wound size was to measure changes from Hour 0 to Hour 8, fly wounds were only visible under the microscope starting at 2 hours after wounding. This is because the melanization of, collection of debris in, and scabbing of larval wounds is what enables their visibility (15). Therefore, percent decreases in wound size were instead calculated from hour 2 to hour 8 after wounding.

Fly mortality was most likely caused by blunt-force injuries to the fruit flies—these flies were observed to have darkened and then died several hours after wounding, a hallmark characteristic of blunt-force injuries in *D. melanogaster* (16). Mortality was most likely not affected by the treatment herbs themselves, as the results found that the differences in mortality between each treatment group and the control group were not significant. In future research, the occurrence of blunt-force injuries can be reduced by using narrower needles, such as needles with diameters ≤ 0.1 mm.

Control group larvae after 2 weeks of culturing

P. somniferum group larvae after 2 weeks of culturing

Figure 4. Control and *P. somniferum* treatment vials depicting larvae. This image of the control and *P. somniferum* culturing vials shows the large number of larvae in the *P. somniferum* vial. This was taken immediately before the removal of 20 larvae for wounding.

Limitations

Both *P.somniferum* and *C.longa* are reported to undergo the first-pass effect upon oral administration. This may necessitate considerably larger oral doses to achieve effects that are comparable to those seen upon topical application, although Curcumin at a dosage of 2 g per day was effective in lowering plasma C-reactive protein levels (17). Unless supplemented in the diet, these compounds are anticipated to be orally ingested after the wound event. This study did not seek to determine whether ingesting these compounds after the wound event would be as effective in shortening the wound time.

Future Research

Future research must be done to confirm the effects of long-term diets containing *C. longa* in wound healing in humans. *C. longa*'s low solubility in water makes it difficult to create medicine containing this herb, but one study has shown that the effects of heat on *C. longa* increase its solubility in water 12-fold, a promising step toward its use not just as a part of a diet, but as a drug in developing areas (18).

Future research may help determine whether the wound-healing effects of *P. somniferum* are related to the trace opium content of these seeds. In addition, one intriguing characteristic of this herb was noted. During culturing of the *P. somniferum* group flies, many more larvae were found in their culture, despite the exact same number of parent flies of each sex. Figure 4 shows the larvae found in the *P. somniferum* vial. This may suggest reproductivity benefits of *P. somniferum* diets. Poppy seed oil may boost fertility when flushed through human fallopian tubes in hysterosalpingography, as one 2022 study finds, but future research is needed (19). Research assessing the effects of various concentrations of *P. somniferum* food medium on amount of *D. melanogaster* offspring may open new doors for a potential herbal infertility treatment.

Conclusion

Overall, this study determined that diets containing *C. longa* and *P. somniferum* significantly shortened the time to heal wounds in *D. melanogaster*. Since the mechanism and signaling pathways for wound healing are substantially similar for both invertebrates and vertebrates, the results of this study may be translatable to humans. Research on the oral applications of these compounds should seek to determine the wound healing biological pathways for optimal diet supplementation, and their effectiveness when administered orally after the wound event. These ubiquitously cultivated and available compounds may hold promise as oral wound healing medicines or supplements in developing regions lacking physical and monetary access to approved wound healing drugs.

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