Effects of Guava Leaf Extract on the Microsocopic Features and Healing Rate of Incisional Wounds in Mice

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Abstract

Wound care and treatment is crucial in hastening prompt recovery and healing of damaged skin or tissues. A wound healing effect was studied in vivo by clinical and histological evaluation in laboratory mice treated with guava leaf extract, povidone iodine solution and normal saline substances. Eighteen BALB/c mice were randomly divided into three treatment groups with six animals per treatment. Each animal served as replicate. Group I was treated with pure guava leaf extract, group II with povidone iodine, and group III, the control group, received plain normal saline solution. Each mice received daily applications of the medications tested. Animals were sacrificed and euthanized after 9 days. Tissue section stained with eosin and hematoxylin were examined and analyzed. Parameters on inflammatory cells, neovascularization, fibroblastic proliferation and epithelial regeneration and presence of red blood cells (RBCs) were evaluated through histological scoring system using the scale of 1-4. 1 for "none", 2 (slight/few), 3 (moderate) and 4 (abundant). In vivo, histological data showed that neovascularization, fibrosis, and epithelialization were found to be substantially increased, an indication of a progressive healing following administration of guava leaf extracts. Inflammation was negligible in all guava leaf extract treated animals but with decreased RBC count, an indication of wound healing process conceivably occurring. Although those on the povidone iodine group yielded an observably a good or fair number of neovascularization, fibrosis and epithelialization and presence of RBCs, inflammatory cells are still high, an indication of prolonged healing process. Histologic data on guava leaf extract provided a higher range of healing efficacy with that of the commercial povidone iodine product. Overall findings suggest the effectiveness of *P. guava* leaf extract as compared to povidone iodine and superior in accelerated wound healing. With this, the "community" at the heart of this study has the premise of choosing better alternative low-cost medicines with equal potency with those synthetic preparations available in the market.

Keywords guava, wound healing, antiseptic, healing rate, low-cost medicine.

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INTRODUCTION

Medicinal plants contribute significantly to primary health care in many countries and serve as the starting point for several semi-

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synthetic analogs. Numerous plants and plant components have demonstrated anti-inflammatory and wound healing properties which illustrates the potential for novel agents to be identified from uncharacterized natural plant resources (Fernandez et al., 2010).

The guava tree, *Psidium guajava Linnaeu*s (Myrtaceae) (hereafter referred to as guava), is a tropical hardwood plant that can reach a height of 10 m. Itis one of those traditional medicinal plants used for treating wounds. Thriving in all types of soils, it is native and indigenous to the Philippines and can be found in almost all places in the country with a long history of traditional use (Adao, 2016). Guava is also used medicinally in many parts of the world as an anti-inflammatory and antiseptic. The leaves are applied to wounds, ulcers and joints (for the relief of rheumatic pain) and are also chewed to relieve toothache (Cheng, 2011).

The wound healing effects of guava had been evaluated in several studies proving its effectiveness, a good proportion of which have been validated by scientific research (Kiran, *et al.,* 2015). However, in a real rural health community setting povidone iodine had been the most popularly used commercially based antimicrobial formulation for wound healing given especially for fresh, uninfected wounds. Local management of wounds that are considered to have potential for healing remains elusive (Woo KY, 2014).

Povidone iodine have remained popular due to their favorable efficacy and tolerability. Its broad spectrum of activity, ability to penetrate biofilms, lack of associated resistance, anti-inflammatory properties, low cytotoxicity, and good tolerability have been cited as important factors, and no negative effect on wound healing has been observed in clinical practice.

Despite of the availability of medications offered in Rural Health Centers, most often than not, patients prefer to simply stay at home and care for themselves particularly when wounds are simply uninfected and do not require complicated treatments. Others who lack the resources to buy medicines and have no access to medical treatments, choose to stay at home and resort to local herbs and plants for treatments (Novaes *et al.,* 2016).

This study is an offshoot of the study conducted on the evaluation of the wound healing potential of guava leaf extracts and commonly used povidone iodine solution focusing on macroscopic clinical observations in terms of wound contraction, inflammation, swelling, elevation and hyperemia, weakness, inappetence and incoordination, the appearance of pus, scab and scar formation wherein guava leaf extract caused an accelerated positive effect on wound healing compared to povidone iodine solution (Delorino et al., 2019).

Specifically, this study determined and compared histologic and microscopic findings in wound healing efficacy of guava leaf extract based on occurrence of inflammatory cells, presence of red blood cells, neovascularization or angiogenesis, epithelial regeneration, and fibroblastic proliferation. Results provided data as inputs for community health education program.

METHODOLOGY

All the materials needed for the study were brought into the facilities of the University of Eastern Philippines College of Veterinary Medicine (UEP-CVM). Commercially prepared povidone iodine (10% solution) and distilled water/plain NSS were procured from a drugstore. Processing of guava leaf extract and the induction and treatment of experimental wound incision to laboratory mice was done at the UEP-CVM laboratory facility. The same procedure and process of

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wound application of the treatments was followed for all animals under study.

Young guava leaves were collected within the vicinity of the University of Eastern Philippines main campus. Soon after collection, the leaves were washed thoroughly in a running tap water and placed in a strainer to drain the water. Extraction of 50 grams of young leaves was carried out using a mortar and pestle to obtain a liquid form of extract of 20 ml. The extract was filtered using a mesh or muslin cloth. Extracted guava juice was placed in a sterile container and stored in the body of the refrigerator to preserve the freshness and the active particles present in it. This was only opened upon use. To assure that the extract is fresh, as much as possible, a daily collection and extraction of leaves was done. Pure guava leaf extract (GLE) treatment was given once a day simultaneous with povidone iodine (PI 10%) *Betadine* solution and plain normal saline (PNSS) or distilled water.

BALB/c laboratory mice of either sex having a homogenous characteristics weighing approximately 20-30 grams from 4-6 weeks old were purchased from the University of the Philippines College of Public Health Central Animal House and were used for the study. They were taken from UP Manila animal research laboratory, housed in cages individually and maintained under standard conditions at the University of Eastern Philippines College of Veterinary Medicine Laboratory Room. They were fed on standard pellet with water *adlibitum* maintained at 12 hours of light and 12 hours dark cycle for 12 days in the departmental laboratory for acclimatization. All the mice were assessed for physiological parameters and only those apparently healthy were considered fit for surgical procedure, thus were considered in the study.

Animals were divided into three (3) groups, consisting of six (6) mice each. Every group were housed individually in the laboratory cages. They were given free access to standard feeds and drinking water and were maintained on a 12-hour light/dark cycle.

Group 1: This were treated with pure guava leaves extract (100%).

Group II: This were treated with standard povidone iodine (10%) Betadine solution.

Group III: This were treated with plain normal saline (PNSS) and served as the placebo or control group.

Povidone iodine solution (Betadine at 10% solution) commercially prepared and obtained from a drugstore was used as one of the treatments in this study. A small amount of povidone more or less enough to cover the wounded area with the use of a dropper was the method used. A cotton swab was also used to cover the wounds at approximately 2cm around the surroundings of the wound following manufacturer's recommendation. After applying the treatment, wounded area was left uncovered since it is difficult to cover it with sterile bandage. There is always the tendency that the mice will remove the dressing. Treatment was given once a day in the morning. A plain NSS or distilled water was purchased from a drugstore and was used as treatment for the placebo or control group.

This study was laid out in a single factor completely randomized design (CRD) with balanced replication. A total of eighteen (18) BALB/c mice were randomly divided into three (3) treatment groups with three (3) animals in each group. Each animal served as a replicate. Replicate Treatment zero (RT0) served as the untreated/negative control group given with distilled water. RT1 and RT2 were the positive control groups. RT1 was treated with pure percent guava leaf extract, and RT2 was treated with povidone iodine (Betadine) at 10% solution.

Anesthesia and Wound Creation

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Surgical procedure was aseptically performed in the animal facility operating room of the College of Veterinary Medicine. Eighteen (18) mice were divided into three (3) groups with six (6) laboratory animals in each group. All animals in each group were anesthetized by intraperitoneal (IP) injection of Zoletil 50 at .01 ml dosage before the incision. A 2 cm full-thickness skin wound was incised from the dorsum of the laboratory mice using a number 10 scalpel blade. Incision was made through the skin and cutaneous muscle at 2 cm from the midline on one side of the depilated back of the mice aseptically.A single incision was made to each of the eighteen (18) mice. On the other hand, a ruler (caliper) was used as a guide on the incision length. Hemorrhage of wound was controlled by compression using sterile gauze. The wound incision was left unsutured for secondary intention healing for 24 hours. The day of wound creation marked the day 0 of the experiment. Application of the formulated test agents commenced after 24 hours post-wounding (Kulkarni, 2001).

The first group received pure guava leaf extract, the second group with povidone iodine (Betadine) at 10% solution, and the third group were given placebo PNSS (distilled water). The mice were observed daily for macroscopic feature changes. The treated area were measured and observed for evidence of gross healing in terms of length and width of wound, inflammation in terms of wound swelling, elevation and hyperemia, presence of clinical signs such as weakness, inappetence, and incoordination, and appearance of pus, scab and scar formation.

Histologic Grading of Wound Healing

After the specimen were observed for gross healing, 9 animals were sacrificed for histologic examination. Following the principle of 3 Rs (Replacement, Reduction and Refinement) for a more human research, 3 animals per treatment were randomly selected. A 0.5 cm biopsy specimen was taken from the wound edges of each representative mouse on its designated groups on days after 9 days. The specimens were fixed in a 10% buffered formalin solution and samples were sent for processing to a private pathology laboratory at University of the Philippines, Los Baños, Laguna. The section of the skin samples was stained with eosin and hematoxylin and examined microscopically under low power to high power objective.

Parameters on inflammatory cells, angiogenesis (neovascularization), fibroblastic proliferation (fibrosis) and epithelial regeneration (epithelization) and presence of red blood cells (RBCs) were evaluated using the Abramov's histological scoring system which is a modified Greenhalgh's scoring system. Parameters scored and graded as: 1 (none), 2 (slight/few, 3 (moderate) and 4 (abundant). RBCs present means outside the blood vessels or capillaries.

RESULTS AND DISCUSSION

The results of the study which determined the microscopic wound healing efficacy of guava leaf extracts and povidone iodine on the incised skin of laboratory mice subjected to histopathological examination are presented in this chapter.

Histopathologic data result on tissue examined

Table 1 shows the group of mice treated with pure GLE. Apparently, mean score of 1 which means "none" presence of inflammatory cells was found in the tissue examined. The RBCs were found to be slight/few (2.33) with a moderate neovascularization (3), a slight epithelial regeneration (2) and a moderate fibroblastic proliferation as shown in Figure 1.

Table 1. Histologic aspect of the tissue samples treated with guava leaf extract and its descriptive analysis

Slide No.	Mice No.	Inflamma-tory Cells	RBC	Neo Vascularization (Angiogenesis)	Epithelial Regenera-tion	Fibroblastic Proliferation (Fibrosis)
8	2	1	4	3	2	3
5	5	1	2	3	1	2
7	6	1	1	3	3	4
Mean score		**1**	**2.33**	**3**	**2**	**3**

Slide No.	Mice No.	Description
8	2	RBCs are numerous; Abundant neovascularization; moderate fibroblastic proliferation; and slight epithelial regeneration; scab formation
5	5	There is much scab formation; slight fibroplasia; moderate neovascularization and slight RBCs present
7	6	There is moderate neovascularization and abundant fibroblastic proliferation with moderate epithelial regeneration; scab formation

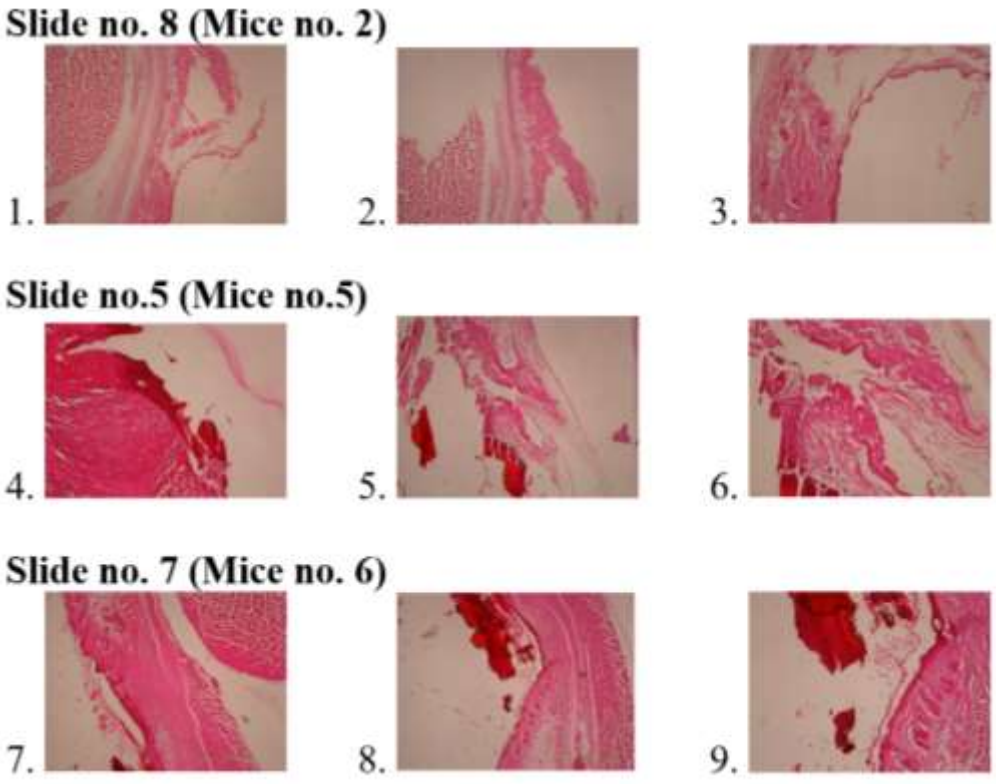

Figure 1. Histopathologic aspect of mice Nos. 2, 5 and 6 treated with guava leaf extract (GLE)

As shown in Table 2, histopathologic samples examined for wound healing in mice treated with Povidone Iodine solution exhibited mean scores of 2 signifying a "slight/few" presences of inflammatory cells. The occurrence of RBCs is "moderate to abundant" having 3.67 mean. For both neovascularization and fibroblastic proliferation, 2.67 mean scores interpreted as "slight o moderate" were noted while parameter on epithelial regeneration appeared to be "slight/few" (1.67 mean score). These all are reflected in the sample slides in Figure 2.

Table 2. Histologic aspect of the tissue samples treated with Povidone Iodine (PI) and its descriptive analysis

Slide No.	Mice No.	Inflammatory Cells	RBC	Neo vascularization	Epithelial Regeneration	Fibroblastic Proliferation
2	12	4	3	2	2	2
3	9	1	4	3	2	3
6	7	1	4	3	1	3
Mean Scores		**2**	**3.67**	**2.67**	**1.67**	**2.67**

Slide No.	Mice No.	Description
2	12	Moderate RBCs with slight neovascularization and fibroplasia; Epithelial apposition noted; scab formation with inflammatory cells
3	9	More of hemorrhagic with many RBCs without inflammatory cells beneath the layer (looks like a closed lesion in one slide made
6	7	Numerous RBCs; No inflammatory cells present; Epithelial regeneration is slight or starting with moderate fibroplasia

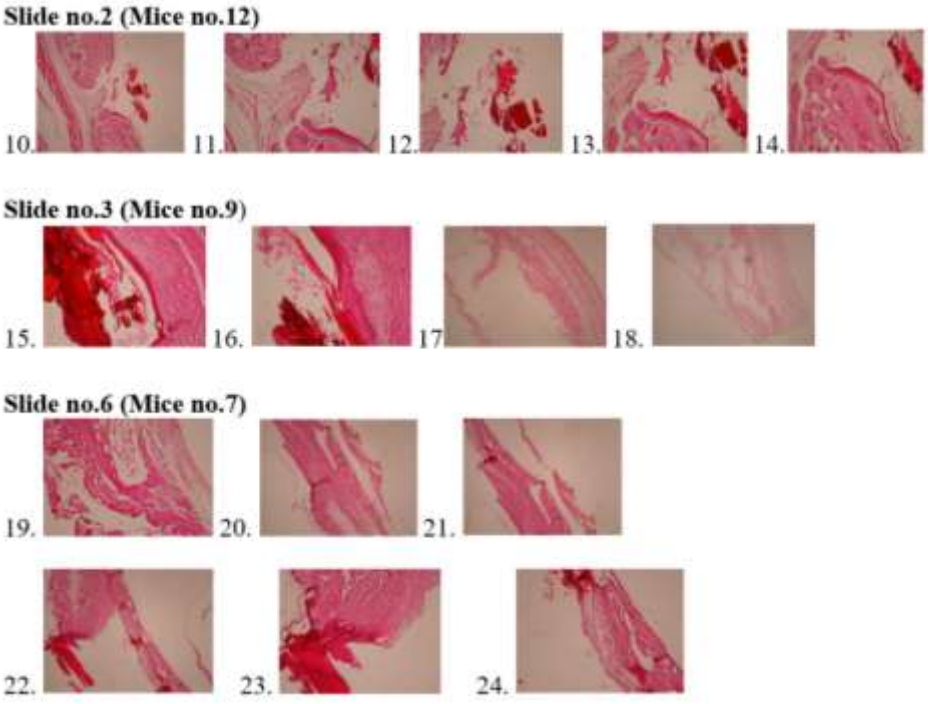

Figure 2. Histological aspect of mice nos.12, 9, and 7 treated with povidone iodine (PI) solution

Histologic data on tissue samples treated with Plain Normal Saline Solution (PNSS)revealed by its mean scores of 2 (slight or few) for presence of inflammatory cells. There were "abundant" RBCs noted, with mean score of 4; a slight to moderate neovascularization with a mean of 2.67 was noted; has "none" epithelial regeneration with a mean score of 1 but with "slight/few" fibroblastic proliferations having a mean score of 2. This are shown in sample slides in Figure 3.

Table 3. Histologic aspect of the tissue samples treated with Plain Normal Saline Solution (PNSS) and its descriptive analysis

Slide No.	Mice No.	Inflamma-tory Cells	RBC	Neo vascularization	Epithelial Regeneration	Fibroblastic Proliferation
1	15	4	4	2	1	1
4	16	1	4	4	1	3
9	18	1	4	2	1	2
Mean score		**2**	**4**	**2.67**	**1**	**2**
Slide No.	**Mice No.**	**Description**				
1	15	There is no epithelial layer present; hemorrhage exudation, moderate neovascularization with slight fibroplasia; scab formation with inflammatory cells present				
4	16	There is much scab formation; many RBCs in the epithelium and neovascularization prominent				
9	18	Almost same as number 2 – Mice no. 2; RBCs are numerous; Abundant neovascularization; moderate fibroblast proliferation; and slight epithelial regeneration; scab formation				

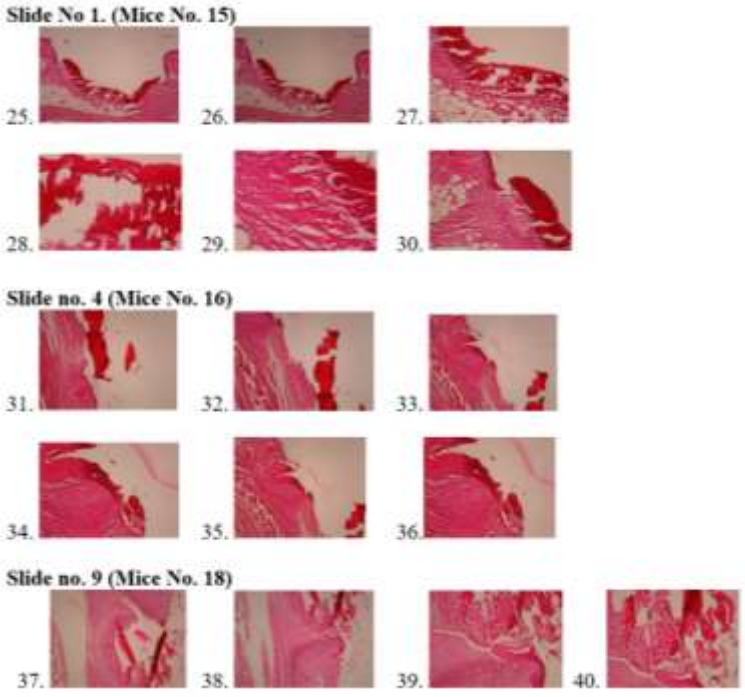

Figure 3. Histological aspect of mice nos. 15, 16, and 18 treated with plain normal saline solution (PNSS)

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Histopathologic data analysis

All animals survived with no complications including infection related to the procedure as observed. Nine days after complete observations, three mice in each group were randomly selected and sacrificed for histologic evaluation. Histological samples were assessed using the Abramov's histological scoring system which is a modified Greenhalgh's scoring system. Parameters were scored and graded as: 1 (none), 2 (slight/few, 3 (moderate) and 4 (abundant). RBCs present means outside the blood vessels or capillaries.

Histopathological findings as presented in Figure 4, were based on mean scores on the microscopic features of tissue samples examined for inflammatory cells, presence of red blood cells (RBCs), neovascularization or angiogenesis, epithelial regeneration, and fibroblastic proliferation.

Comparing the data on presence of inflammatory cells it was found to be highest in specimen treated with PI and PNSS having a mean score of 2, described as "slight or few", while specimen for GLE treatment had a lower mean score of 1 interpreted as "none". The inflammatory phase is an important phase in wound healing process that occurs immediately following the injury and lasts approximately for 6 days. At nine days period inflammatory cells should have disappeared and the fibroblastic phase occurs at the termination of the inflammatory phase and can last up to 4 weeks

On the account of the occurrence of RBCs, it was noted highest in PNSS treatment with a mean score of 4 or "abundant", followed by PI solution having a score of 3 (moderate) and was lowest in GLE treatment with a mean score of 2.33 (slightly to moderate). Red blood cells help create collagen, which are tough, white fibers that form the foundation for new tissue where new skin begins to form over this tissue. The presence of platelets in RBC may influence wound inflammation, but that platelets do not significantly affect the proliferative aspects of repair, including wound closure, angiogenesis, and collagen synthesis. Thrombocytopenia or low platelets count was found to have no significant effect on any of the proliferative aspects of healing. The results suggest that platelets influence the inflammatory cascade at sites of tissue injury, but are not primary participants in mediating wound closure, collagen synthesis, or angiogenesis (Szpaderska *et al.,* 2003)

In the case of mice given with GLE medication, even if there was a decrease in RBC counts, acceleration of wound healing was not affected as evidenced by increased neovascularization, fibroblastic proliferation and epithelization as compared to the two treatments models given with PI and PNSS.

In terms of neovascularization or angiogenesis, epithelial regeneration and fibroblastic proliferation, tissue samples treated GLE had consistently higher mean scores compared to PI and PNSS. It has a mean score of 3 or "moderate" both for neovascularization and fibroblastic regeneration and 2 (slight/few) for epithelia regeneration. PI treated group obtained much lower mean scores both for neovascularization fibroblastic regeneration with of 2.67(few-moderate) mean scores and a much lower score of 1.67 (few) for epithelial regeneration. Those treated with PNSS got a similar mean score with PI treated animals in terms of neovascularization with mean score of 2.67 (few to moderate) but much lower to PI treatment in fibroblastic proliferation with mean score of 2 interpreted as "few/slight" while there was "none" epithelial regeneration that occurred, having a mean score of 1.

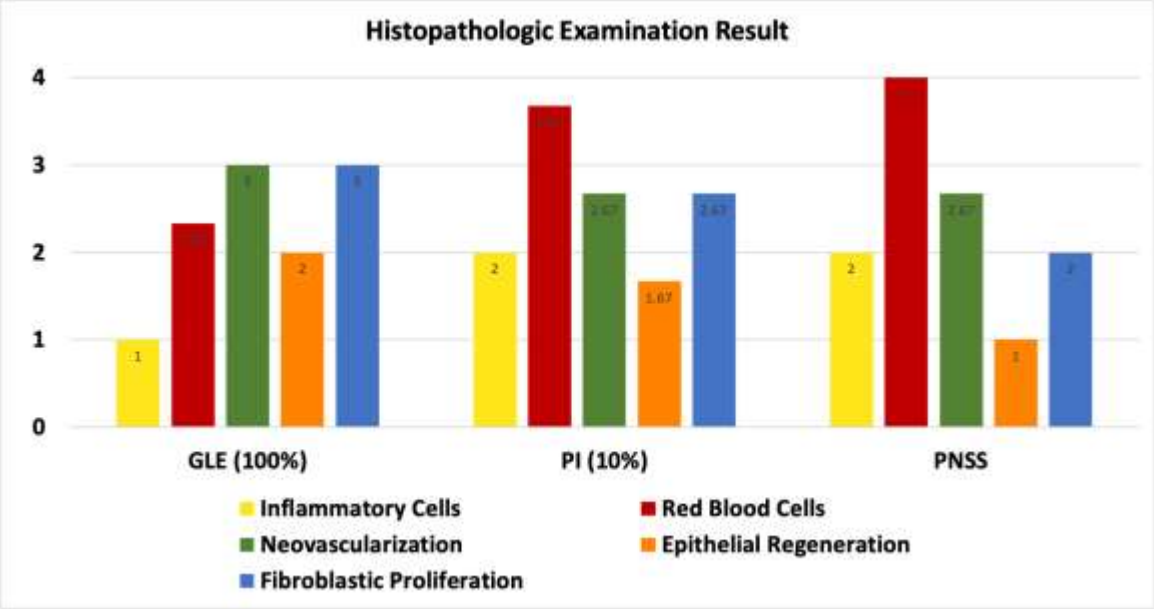

Figure 4. Histopathological scores among groups at Day 1 to Day 9. Inflammatory cells, Red Blood Cells (RBCs), neovascularization, epithelial regeneration, and fibroblastic proliferation of wounds in mice treated with Guava Leaf Extract (100%), Povidone Iodine (10%solution), and Plain Normal Saline Solution (PNSS).

LEGEND 1 – None 2 – Slight/few 3 – Moderate 4 – Abundant

• RBCs present means outside the blood vessels/capillaries

CONCLUSION

Conclusively, histopathologic data provided a higher if not similar range of healing efficacy for GLE than PI solution. Overall findings suggest the effectiveness of *P. guava* leaf extract as compared to povidone iodine as higher or more superior in wound healing. Therefore, "community" being at the heart of this study has the premise of choosing better alternative low cost medicines with equal potency with those synthetic preparations available in the market. Hence proper conservation and sustainable use of guava plant resources and the incorporation of the traditional knowledge with scientific findings should be enhanced through community health education.

CONFLICT OF INTEREST

The researcher declares no conflict of interest.

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