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EFFECT OF GUAVA (*Psidium guajava*) CREAM ON THE GROSS AND MICROSCOPIC FEATURES AND HEALING RATE OF INCISIONAL WOUNDS IN DOMESTIC SHORT-HAIRED CATS

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ABSTRACT

The study was conducted to investigate the feasibility of guava cream as replacement for topical antibiotic for wound healing. Twelve cats were divided into four groups and subjected to different concentrations of guava cream: 0% (antibiotic wound cream group), 1%, 3% and 5% having 3 animals per group. A dorsal midline incision (at the level of T6 to T7, with a length of 2.5 centimeters in full thickness) was made and was left open for second intention healing. A thin layer of cream was applied over the wound every other day starting day 0 until day 20 and an incisional biopsy was done at day 0, 7, and 14. Wound contraction and gross appearance was graded. There was no significant difference (P<0.05) on the effect of wound healing of the different percentages of guava cream compared to the wound cream however, the results of the 3% and 5% guava cream had a faster wound contraction. All treatments of guava cream produced comparable results with the antibiotic wound cream therefore, this study concludes that guava cream can be used as an alternative antiseptic and antibacterial treatment for wounds.

Keywords: feline, guava cream, *Psidium guajava*, wound healing

INTRODUCTION

Incisional wounds are very prone to contaminants such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium* sp., *Escherichia coli* and *Pseudomonas aeruginosa* (Abubakar, 2009) which are factors that delays the healing process by infecting the wounds which results in impedance of proper tissue repair.

Psidium guajava, the popularly known "guava" or "bayabas" in the Philippines, has a high potential as an alternative treatment for wound healing and its price can be cheaper due to its abundance in the area (Vieira, 2001). The leaves are applied to wounds, ulcers and joints for the relief of rheumatic pain and are also chewed to relieve toothache (Gutierrez, 2007).It can be seen in a Dutch Pharmacopoeia as a legitimate herbal treatment for several diseases including rheumatism, cough, vaginal disorders, anti-spasmodic, and more (Kumar, 2012).

The leaf extracts of *P. guajava* has antimicrobial effects against bacteria that are commonly present on wound surfaces specifically surgical wounds (Biswas *et al*., 2013). Scientific basis on the utilization of crude extracts from guava was established in herbal medicine (Gutierrez, 2008). The full identification of the pharmaceutical effect of the drug is still dependent upon the purification of the biologically active components present in the plant. Its full potential can only be reached upon isolation of these active components. Chemical compounds present in the *Psidium guajava* like tannins, polyphenolic compounds,

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flavonoids, ellagic acid, triterpenoids, guiajaverin, quercetin, and other are believed to account for the observed anti-inflammatory and analgesic effects of the plant's leaf extract (Abubakar, 2009). In the study made by Wang *et al.* (2014) the anti-inflammatory property of the aqueous leaf extract was investigated using fresh egg albumin-induced pedal edema in rats. Also in the mice, the analgesic effect of the plant extract was studied by using "hot-plate" and "acetic acid" test models of pain. This study aims to test which concentration of guava extract most efficacious in hastening the wound healing process to be used as an alternative topical wound cream.

MATERIALS AND METHODS

All experimental procedures conducted in this study were permitted and approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of the Philippines Los Baños.

The study employed twelve (12) domestic short-haired cats from San Pablo City, Laguna. The samples were limited to apparently healthy male animals with a body condition score ranging from 4/9 to 5/9, weighing of 2-3 kilograms and were between six months to two years of age. After the acquisition of the animals, they were subjected to thorough physical examination and a complete blood count was done to remove underlying factors that might affect the results of the experiment. Only cats that are inside the reference values of the WBC, RBC, PCV, and platelet counts that were based on Plumb's Veterinary Drug Handbook (Plumb, 2015) were accepted. All selected cats were placed in a confined room in separate cages for a week for acclimatization before experimentation. Each were fed 150 g of commercial cat food daily and were provided with water *ad libitum*.

A randomized controlled trial was done for the designation of cats in each treatment group and three cats were assigned under each treatment. Four treatment groups were used in this experimentation namely: 1) a topical antiseptic and antibiotic wound cream (Topi-derm® VetMate, Quezon City, Philippines); Ingredients: iodine 5%, co-trimazine 0.5%, sulfur 7%, borax, guava leaf extract, and eucalyptus oil with cream base as carrier. 2) 1% guava cream, 3) 3% guava cream, and 4) 5% guava cream. These creams contain guava leaf extract with different percentages with cream base as carrier (Guava cream, prepared by Marklene Fabricate Corporation, Bulacan, Philippines).

All cats were injected with Tiletamine HCl – Zolazepam HCl (Zoletil®, 50mg/ml, 5mg/kg) for general anesthesia prior to inducing incisional wounds. The area of incision was shaved using a clipper and was disinfected with alcohol and povidone-iodine. A number 10 scalpel blade was used to generate incisional wounds to each of the cats where in one incision was made to each of the twelve cats. The incision site was on the dorsum at the level of T6 to T7 having a length of 2.5 centimeters in full thickness. The wound was left for second intention healing and no patches were applied.

All cats under their designated groups were treated once every other day starting day for 20 days. All wounds were washed with normal saline solution before cream application; sterile cotton swabs were used as an applicator with an amount approximately 0.5 mL of the assigned cream.

Macroscopic grading on the wounds under all treatment groups was made every other day starting day 0 until day 20. The independent blind scoring was based on the method of Chupeco *et al.* (2013). All data were recorded and photographed for documentation and comparison.

Evaluation on the contraction of wounds:

The degree of wound contraction was measured using the method of Agarwal *et al.* (2009) on the whole duration of the experiment. The % wound contraction was computed based on the equation below:

% wound contraction =(Healed area/Total wound area (2.5 cm)) x 100

where: Healed area= Total wound area – present wound area,

Total wound area= Length x width of wound at day 0 (2.5 cm)

Present wound area= Length x width of the wound at the day of observation

Wounds of each cat under different treatments were observed and recorded every other day starting day 0 up to day 20. The initial length and width of the incisional wound of each cat were recorded after the surgery was made. This was used as the baseline value for the computation of the percent wound contraction. The percent wound contraction was computed by dividing the healed area over the total wound area then multiplied by 100. The healed area was derived by subtracting the total wound area to the present wound area. Computation of the wound area was done by multiplying the length of the wound to its width. In the case of those cats that had biopsy, total wound area was not divided by 2.5 centimeters but by the length of the wound observed after biopsy. The area where biopsy was made was negated.

Histological section of the wounds was graded using the following:

Representative animals were subjected to incisional biopsy at day 7, and day 14 for histologic grading of parameters. One cat in each treatment was randomly selected as the representative of the group. The area was first disinfected with povidone-iodine before the procedure was performed. A section of the wound about 0.2 to 0.4 centimeters was taken for histopathologic evaluation using a number 10 scalpel blade for biopsy. Tissue near the edge of the wound was excised and taken. It was placed in a sterile cup with 10 mL of 10% buffered formalin. The wound from the incisional biopsy was sutured using a chromic catgut suture material (Ethicon®, Johnson and Johnson, Parañaque, Philippines) and a cruciate suture pattern was made to close the area. Hematoxylin-eosin (Fluka®, Fluka Chemie AG, Germany) was the stain used for the histologic analysis of the skin sample. The skin samples collected were viewed under the microscope (Nikon® Eclipse E200, Nippon Kogaku KK, Japan) using a low power and high power objective. All observations were recorded and comparisons between the days of healing and between treatments were noted. Histological samples were assessed based on the method of Nisbet *et al.* (2010) which is a Modified Greenhalggh's scoring system.

Statistical analysis were applied on the results obtained on the set of parameters used. The variations within the differences of mean score between treatments were analyzed using Kruskal-Wallis test and One way ANOVA for the difference between means per treatment day ($P<0.05$).

RESULTS AND DISCUSSION

Table 1 shows the mean ±SD on the percent wound contraction on each treatment from days 2 to 20. Increasing percent wound contraction can be observed from days 0 to 20 except at day 4 where in there was a decline in the percentage of wound contraction. For the 1% guava cream, the values rapidly increased from day 0 to day 6 but declined at day 8 and slowly increased up until day 20. For the 3% guava cream, day 0 started with a significantly high percentage of wound contraction compared to that of the 1% and the 3% guava cream. The values obtained also showed increasing values from day 2 to day 20. For the 5% guava cream, the data was similar to that of the 3% guava cream except that the percent wound contraction in the first 3 days started low. All wounds gain strength at approximately the same rate during the first 14–21 days. Skin apposition and wound healing could be affected by different factors such as mechanical stress, infection, and presence of foreign body. Mechanical stress on the healing wound affect the quantity, aggregation, and orientation of collagen fibers (Forrester, 1970).

In Figure 1, signs of mild inflammation, exhibited by redness and swelling, were immediately seen after wounding. After a week, inflammation subsided and scabbing started to be observed. Granulation tissue was observed on the 3% guava cream treatment but the rest did not display exuberant granulation. Reactions such as edema, wound vesiculation, or marked accumulation of exudate were not observed. The results on different treatments of topical cream showed increase in the rate of wound healing of the incisional wound in each of the cats. The 3% and 5% guava cream showed a remarkable rate on the wound healing in terms of the reduction of the wound size and the prevention of contamination. It could be comparable with the wound cream while the 1% guava cream showed a fair result in the aspect of wound reduction. From day 0

Table 1. Mean±SD of the percent wound contraction on the skin of animals treated with a wound cream and different concentrations of guava cream.

Day	Treatment			
	Wound cream	1% guava cream	3% guava cream	5% guava cream
2	13.3±0.00	0.0±0.00^{b}	13.3±0.00	7.4±0.00
4	4.8±0.00	34.4±0.00^{a}	18.9±0.00	23.7±0.29
6	11.4±0.00^{b}	45.6±0.00^{a}	27.7±0.14	24.6±0.25
8	28.0±0.00^{b}	33.1±0.00	35.6±0.76	37.1±0.14
10	46.0±0.00	42.5±0.29^{b}	52.0±0.50	52.5±0.29
12	72.0±0.18^{a}	60.6±0.63	59.7±0.38	66.1±0.38
14	96.0 ± 0.35^{a}	75.9±0.52	84.7±0.14	81.2±0.52
16	100.0±0.00^{a}	80.5±0.58	93.3±0.58	78.9±0.76
18	100.0±0.00^{a}	89.2±0.63	96.7±0.29	82.6±0.80^{b}
20	100.0±0.00	94.0±0.43	99.4±0.14	93.0±0.52

Means with different superscripts in the same row are different ($P<0.05$).

Fig. 1. Gross appearance of incisional wounds of representative cats treated with a wound cream, 1%, 3%, and 5% guava cream at Day 0, 7, 14, and 20. Distinct wound contraction was observed in all treatments from Day 0 to Day 20.

to day 7 there was a significant decrease in the size of the wound in all treatment groups. At day 14, the wound cream already had complete apposition of the skin. Treatments of 3% and 5% guava cream had complete apposition later than that of the wound cream. In contrast, the 1% guava cream did not show complete apposition on the skin. Infection in the wound prolongs the inflammatory phase of healing while low levels bacteria appear to accelerate wound healing and the formation of granulation tissue (Robson, 1990; Laato, 1988). Foreign bodies, such as nonviable tissue, serve as a hindrance to wound healing and an asylum for bacteria. Similar to wound infection, foreign bodies prolong the inflammatory phase and wound contraction is impeded, repopulate the area with capillaries, or completely epithelialize. Wounds with the presence of necrotic tissue will not heal until all the necrotic tissue is removed (Steed, 2004). In this case, it is possible that the treatment with 1% guava cream experienced one of these challenges which resulted to the partial healing of the wound or the concentration of guava cream was not enough to inhibit these factors. Separate parts of a wound may be at different stages of healing at any one time (Broughton, 2006; Bischoff, 1999; Richardson, 2004; Komarcevic, 2000).

Table 2 shows the mean results of the macroscopically observed data of scab, pus formation, erythema, elevation and scar formation in cats treated with a wound cream,

1%, 3%, and 5% guava cream. The effect of the wound cream on all the parameters tested showed no significant differences between treatments. The 5% guava cream in terms of scab formation is similar to the effect of the wound cream. The 5% guava cream had the least formation of pus compared to the 1% and 3% guava cream from day 12 to day 20. Mbuh *et al*. (2008) investigated the antibacterial activity of the leaf extract of *P. guajava* and reported that the crude extracts inhibited the growth of *S. aureus, S. typhi, E. coli, B. subtilis, Shigella spp., P. mirabilis* and *K. pneumonia.* They concluded that the antibacterial activity of guava cream inhibited the growth of microorganisms that commonly contaminate the

Table 2. Mean±SD scores of scab formation, presence of pus, wound erythema, and scar formation on cats treated with a wound cream and different concentrations of guava cream.

Parameter	Group	Treatment days Mean±SD		
		Day 8	Day 14	Day 20
Scab formation	Wound cream	3.5±2.12	2.0±0.00	2.0±2.82[a]
	1% Guava cream	2.3±0.58[b]	3.0±1.00	1.7±0.58
	3% Guava cream	3.7±1.15	4.3±0.58[a]	1.3±1.53
	5% Guava cream	4.3±0.58[a]	1.0±1.00[b]	1.3±2.31
Presence of pus	Wound cream	1.0±0.00	1.5±0.71	1.0±1.41
	1% Guava cream	1.3±0.58	1.7±0.58	1.0±0.00
	3% Guava cream	2.0±0.00[a]	1.7±0.58	0.7±1.15
	5% Guava cream	1.7±1.53	1.0±0.00	1.0±1.00
Wound erythema	Wound cream	1.5±0.71	1.0±0.00	1.0±0.00
	1% Guava cream	1.3±0.58	2.0±0.00	1.3±1.15
	3% Guava cream	1.3±1.15	2.0±0.00	1.3±0.58
	5% Guava cream	2.0±1.00[a]	1.7±0.58	1.0±1.00
Wound elevation	Wound cream	1.5±0.71[a]	1.0±0.00	1.0±0.41
	1% Guava cream	1.0±0.00	1.3±0.58	0.7±0.58[b]
	3% Guava cream	1.0±0.00	1.0±0.00	1.0±0.00
	5% Guava cream	1.3±1.15	1.0±0.00	1.3±0.58[a]
Scar formation	Wound cream	1.0±0.00	2.0±0.00[a]	2.5±0.71[a]
	1% Guava cream	1.7±0.58[a]	1.0±0.00[b]	2.0±0.00
	3% Guava cream	1.3±0.58	1.3±0.58	2.0±0.00
	5% Guava cream	1.0±1.00	1.7±0.58	1.3±0.58[b]

Means with different superscripts in the same row are different (P<0.05); Scab formation (0-5): 0 for 0%; 1 for 20%; 2 for 40%; 3 for 60%; 4 for 0%; and 5 for 100% of the wound is covered in scabs. Pus formation (0-3): 0 for absent; 1 for slight; 2 for moderate; and 3 for marked presence of pus in the wounds. Erythema (0-3): 0 for absent; 1 for slightly; 2 for moderately pink; and 3 for red areas surrounding the wound site. Elevation (0-3): 0 for absent; 1 for slightly; 2 for moderately; and 3 for markedly raised area surrounding the wound site. Scar formation (0-3): 0 for absent; 1 for slight; 2 for moderate; and 3 for marked scarred area surrounding the wound site.

wound area. Wound erythema had an increasing trend at first, then eventually declined. Bhattacharya (2011) did a preliminary phytochemical study which revealed presence of phenolic compounds in *Psidium guajava* leaf extracts having anti-inflammatory properties. The decreasing trend of the erythema observed on the wound of each cats could be attributed to the anti-inflammatory effect of guava cream. The 5% guava cream had the least percentage of variability compared to the wound cream except for the last day of observation. This is interpreted as there is no significant difference in the effect of the scar formation of the wound cream and the 1% guava cream. These data shows that all concentrations of guava cream could be comparable to the effect the wound cream tested.

A scar is formed by the growth of collagen beneath the skin that which results to wound healing. It may take up to 24 months for the scar to achieve its full strength but it will never be as strong as normal, uninjured skin (Niessen *et al*, 1999). Even after wound apposition and scar formation, there are still internal processes happening on the animal's body to continue the repair of the broken skin. This remodeling phase continues for up to 2 years (Himesh, 2012).

Figure 2 shows the comparison between day 7 and day 14 of observation. Polymorphonuclear cells and fibroblastic proliferation were observed on all treatment groups. At day 14, there was already complete apposition on treatment 1 but the connective tissue were still immature. Collagen formation from day 7 to day 14 increased under all treatment groups but treatment 1 showed the highest increase. These manifestations indicates the acceleration of the wound healing for all treatments (Suguna *et al*., 1992). The 3% guava cream showed the most proximate results compared to the antibiotic wound cream and even had a better performance in angiogenesis and epithelialization.

These histologic changes are evidence of the wound increasing its tensile strength especially when it comes to the increase in the number of fibroblasts observed. This parameter is one of the important indication of the pharmacological effect of the wound healing property of the wound healing agent (Rozaini *et al*, 2005). The presence of macrophages is a good indication that the wound healing is effective. A chemical factor in macrophages is necessary for proper angiogenesis in early wounds (Broughton *et al*, 2005). The healing activity of *Psidium guajava* extract could be attributed to its constituents like quercetin and Zinc. Vitamin.C accelerated the healing process (Mudliar, 2008; Nadkarni, 2000). Nonsteroidal anti-inflammatory drugs (aspirin and ibuprofen) have been shown by Kulick *et al* (1987) to decrease collagen synthesis in an average of 45% even at ordinary therapeutic doses. The effect is dose-dependent and mediated through prostaglandins (Dingfelder, 1982). This could be a possible reason why the values seen in the 5% guava cream is lower compared to the 3% and 1% guava cream.

All treatments of guava cream produced comparable results with the antibiotic wound cream. Observations of a positive effect by guava and wound healing were consistent with other studies. Skin apposition and the wound healing might be affected by the behavior of the cat one of which is that they tend to lick the area where the incision was placed which reduces the contact time of the cream on the wound. It is highly recommended that the wound could be placed somewhere it could not be reached (e.g. dorsal thoracic area).

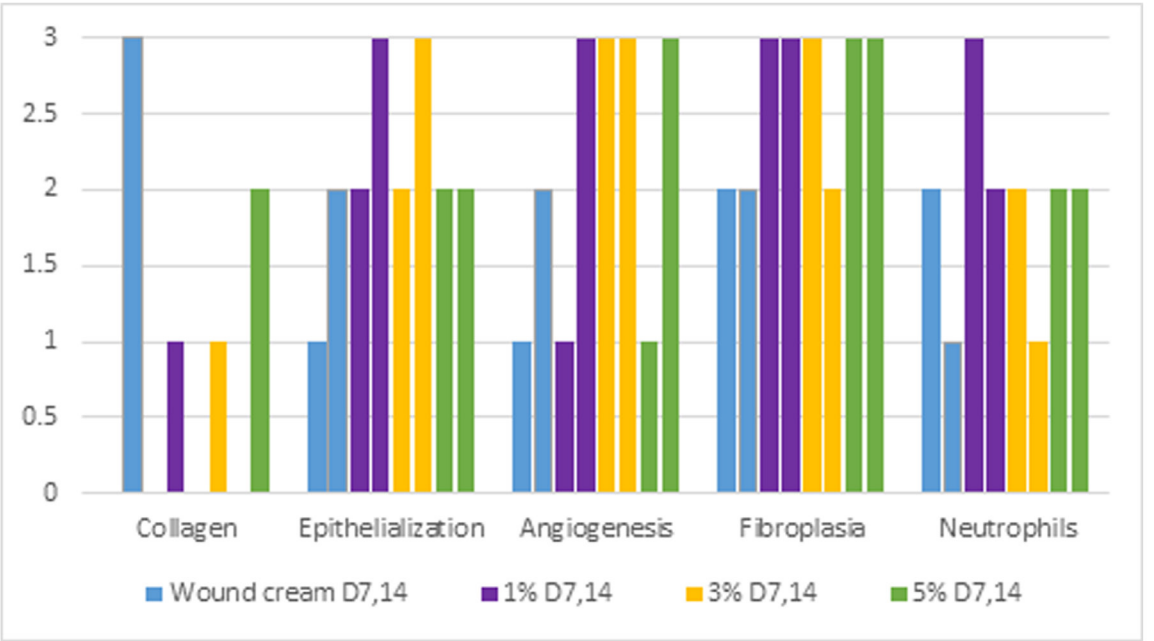

Fig. 2. Histopathological scores among groups at Day 7 and Day 14. Collagen, epithelialization, angiogenesis, fibroplasia, and neutrophils of wounds in cats treated with a wound cream, 1%, 3%, 5% guava cream (Arrangement of bars from left to right; 1st for day 7, 2nd for day 14 in each treatments). The 3% showed nearest values comparable to the wound cream.

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