Evaluation of the Efficacy of a Liquid Dishwashing Detergent in the Treatment of Infected Wound in Sprague Dawley Rats

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***Abstract*— Quality of care for infected wounds is an important requirement in the prevention of systemic infections. Preventive solutions must therefore be evaluated to aid in the care of infected wounds. The study evaluated the therapeutic effect of liquid dishwashing detergent (LDD) on infected wounds through bacterial reduction assay, measurement of the wound closure rate and epithelization in a rat model. The bacterial reduction assay showed a significant difference (p-value=1.06E-16) with 1:10 LDD dilution showing the lowest absorbance. The wound closure rate was comparable to Chlorhexidine (p-value=0.373) at 1:10 LDD dilution. Histopathological samples exhibited neovascularization and fibroplasia as well as the presence of granulation that signifies wound healing.**

***Keywords*— Antibacterial, Epithelialization, Infection, Therapeutic effect, Wound closure rate**

I. Introduction

Wounds, if not treated properly, can often lead to infections. Patients with a weak immune system are often those who develop systemic infections because of untreated wounds. Antimicrobial compounds such as antibiotics are thus essential in treating and preventing the spread of infections. However, it has been well documented that frequent exposure and misuse of antimicrobials can lead to drug resistance which can further cause problems to patients.

A cutaneous wound is brought about by any injury may it be superficial or deep. A healthy intact skin is very essential to fight against pathogens from the environment and for the normal regenerative mechanism to be activated to fix any defect brought about by such pathogens. The primary goal of healing is to restore the preinjured form and function of the skin. There are many factors as to why there are such events like slower or retarded healing due to bacterial infection, lower oxygen tension, systemic problems, metabolic and ageing problems (Cukjati et al., 2001).

Prevention and treatment of infection to increase the rate of healing is the main objective of using antiseptics. Infections leads to delay in the healing process. Microbial contamination may eat up time for wound healing through several different mechanisms, such as metabolic wastes, toxins, and persistent production of inflammatory mediators, and maintenance of the activated state of neutrophils, which produce cytolytic enzymes and free oxygen radicals. Competition between the bacteria and the host cells for nutritional requirement and oxygen for wound healing is also another factor. Wound infection can also lead to tissue hypoxia, render the granulation tissue haemorrhagic and fragile, reduce fibroblast number and collagen production, and damage re-epithelization. Infection or microbial contamination still plays a critical role in wound management. (Drosou A. et al., 2003).

Several wound antiseptics are currently commercially available, one of these is Chlorhexidine which is a known antiseptic and disinfectant. If maintained in a pH of 5 and 8, it is able to retain the best biological activity and chemical stability. It can inhibit most gram-positive and gram-negative bacteria, some viruses, *Candida albicans* and mycobacteria. It has become one of the most commonly used disinfectants in the medical setting since its introduction in 1954. As gluconate salt or acetate, chlorhexidine is mostly used for topical application on skin or mucous membranes, surgical instruments, wounds, burns, and surfaces. However, irritant contact dermatitis is a common adverse reaction to chlorhexidine (Krautheim A. B. et al., 2004). This problem serves as the basis for the researchers to look for alternative wound antiseptics that is less harmful to the skin and can probably lead to wound healing. The suggested alternative is the use of commercially available liquid dishwashing detergent that claims to have antibacterial action. This study will assess its potential as antiseptic and wound healing effect in terms of bacterial reduction, wound closure rate and epithelization.

II. Methods

A. Research Ethics

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University. A permit from the Bureau of Animal Industry was also obtained with the reference number AR-2016-179. The test animals were kept in conditions in line with the guidelines set forth by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were kept in steel cages and maintained under standard housing conditions of

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temperature (24-27°C) and humidity (60-65%) with a 12 h light–12 h dark cycle. They were acclimatized for seven days. Food was provided in the form of dry pellets and water ad libitum.

B. Bacterial Suspension

Pure culture of *Staphylococcus aureus* was purchased from the culture collection department of University of Santo Tomas. The bacteria were maintained in Nutrient broth medium. It was then subcultured to Mannitol Salt Agar. Golden yellow colonies was picked and suspended in a distilled water to obtain a concentration of 10^9 bacteria per ml of suspension. It was measured by means of spectrophotometry at 625nm.

C. Excision wound model

The rats were anesthetized prior to and during the creation of the wounds, with 1 ml of intravenous ketamine hydrochloride (10 mg/kg) (Dash G. K. 2012). Following anesthesia, the animals were immobilized and placed on a wooden board, protected by a one cm-thick Styrofoam sheet and tied on their paws and legs with rubber bands in the horizontal position lying on their bellies (J'Acampora et al.,2006). The dorsal fur of the animals was shaved with an (Schick Disposable) Twin Blade Razors. The wound site was measured and marked at about 2.5 cm diameter, using a skin marker . A full-skin thickness wound was created at the marked site by excising the skin flap with sterile scissors and forceps (Padol AR., 2012).

After achieving complete haemostasis by blotting the wound with cotton, the wound of each animal in Group I, II, III, IV and V underwent topical application of 109 bacteria per ml of suspension (J'Acampora et al.,2006) and wounds were left undressed. All surgical procedures were performed under aseptic conditions. After 4 days of wounding the wound were cleansed twice daily with their assigned treatment from day 5 until the end of the study (Mendes J.J. et al., 2012). Wound closure rate were assessed by measuring the wound on days 1, 4, 6, 8, 11, 14 and 16 post wounding days using vernier caliper. The wound areas measured were recorded.

D. Bacterial reduction assay

To standardized the inoculum density used in the study, the bacterial suspension is compared to 0.5 McFarland standard. Direct colony suspension method is performed by making a direct saline suspension of isolated colonies of *Staphylococcus aureus*. Suspension turbidity compared to 0.5 McFarland standard results in a suspension containing approximately 1 to 2x108 CFU/ml. The researchers used spectrophotometer with a 1cm light path; for accurate reading of bacterial suspension (control strain) and 0.5 Mcfarland standard, the absorbance at a wavelength of of 625nm should be 0.08 to 0.13 (CLSI).

In vitro testing used five groups with five test tube in each group, Group I served as negative control (500ul of tap water added), Group II served as positive control (500ul of chlorhexidine solution), Group III served as experimental group (500ul of 1:1 dilution of Liquid Dishwashing Detergent), Group IV served as experimental group (500ul of 1:10 dilution of Liquid Dishwashing Detergent) and Group V served as experimental group (500ul of pure Liquid Dishwashing Detergent). Each tubes contain 2000ul of Mueller-Hinton Broth and 500 ul of bacterial suspension. The tubes were incubated for 24 hours and using spectrophotometer the absorbance at a wavelength of of 625nm is read after incubation period.

E. Epithelialization

Tissue samples was obtained after the treatment cycle. Hematoxylin and eosin staining was used to observe the healing process in a tissue level. The presence of scab, re-epithelization, tissue granulation, neovascularization, fibroplasia and mononuclear infiltrates was noted.

F. Data Analysis

The values were calculated as mean ± S.E.M. Significant difference was tested using one way ANOVA. It was calculated using Graph pad prism.

III. Results and Discussion

Wound is a complex phenomenon causing destruction or rupture or discontinuation of tissue in a particular part of the body with discoloration. Due to this prevalence of wound infection, we come up to this study, producing an alternative way to reduce the infection of wound brought by bacteria, this study provides the information about the effectiveness of liquid dishwashing detergent in treating infected wounds. In our model, Sprague Dawley rats was excised and infected with Staphylococcus aureus. We grouped our rats into five, where group one treated with tap water, group two treated with chlorhexidine (positive group), group three treated with diluted liquid dishwashing detergent with a concentration of 1:1, group four treated with diluted liquid dishwashing detergent with a concentration of 1:10 and lastly group five treated with pure liquid dishwashing detergent.

Wound closure rate was done to assess the wound healing effect of LDD. The significant difference in the wound closure rate of negative control (Tap Water group) compared to Liquid Dishwashing detergent is summarized in Table 1.0. A significant difference was observed in 1:10 dilution of LDD. While a significant difference was observed in concentrated LDD when compared to Chlorhexidine.

TABLE 1.0 COMPARISON OF WOUND CLOSURE RATE

Groups	Average	Variance	T-test	P-value	T- test critical	Decision	Intrepretation
Tap Water vs LDD 1:1	0.0984375 0.0984375	3.58073E-05 0.000117188	-1.28368	0.240110429	2.364624	Accept Ho	Insignificant Differrence
Tap Water vs LDD 1:10	0.0984375 0.13125	3.58073E-05 1.95313E-05	-9.50069	2.99567E-05	2.364624	Reject Ho	Significant Difference
Tap Water vs LDD concentrated	0.0984375 0.105	3.58073E-05 0.000046875	1.507157	1.894578604	2.364624	Accept Ho	Insignificant Differrence
Chlo vs LDD 1:1	0.1325 0.13125	2.73437E-05 0.000117188	7.202941	0.001969111	2.776445	Reject Ho	Significant Difference
chlo vs LDD 1:10	0.1325 0.13125	2.73437E-05 1.95313E-05	1	0.373900966	2.776445	Accept Ho	Insignificant Differrence
chlo vs LDD concentrated	0.1325 0.105	2.73437E-05 0.000046875	17.96292	5.64577E-05	2.776445	Reject Ho	Significant Difference

*LDD – Liquid Dishwasing Detergent *chlor - Chlorhexidine

It was noted that only the diluted liquid dishwashing detergent with a ratio or concentration of 1:10 (LDD 1:10) had similar effect as the positive control which is treated with chlorhexidine. Although wound closure was observed, the effect of LDD is attributed only to its antimicrobial content. The antimicrobial action was able to inhibit the growth of bacteria which helped in the wound healing process.

Bacterial reduction assay was based on its optical density (absorbance), Spectophotometer with 625 nm wavelength was used to measure this parameter. It can be observed in Table 2.0 that there was an increased absorbance of the negative control which is the tap water and a decreased values of the absorbance of the three different concentration of liquid dishwashing detergent. Detergents when diluted will result in a low detergent to microbial lipid ratio. This often leads to temporary stabilization in microbial membranes. However, openings might form temporarily at intermediate concentrations, and microbial membrane disintegration occurs at higher antimicrobial peptide-to-lipid ratios. This can lead to loss of their membrane barriers which leads to loss of cytoplasmic constituents which can hinder energy metabolism of living microbial cells (Bechinger and Lohner 2006).

The growth of bacteria incubated with the negative control, LDD and Chlorhexidine was compared and summarized at Table 3.0. If compared to the negative control, LDD has a lower density of bacteria. This is suggestive of its inhibitory effect in bacterial growth.

TABLE 2.0 MEAN ABSORBANCE OF STAPHYLOCOCCUS AUREUS SUSPENSION

Groups	Mean
tap water	0.3856
CHLORHEXIDINE GRP.	1.3148
1:1 LDD GRP.	0.1862
1:10 LDD GRP.	0.1472
CONCENTRATED LDD GRP.	0.161

*LDD- liquid dishwashing detergent

TABLE 3.0 COMPARISON OF BACTERIAL REDUCTION EFFECT OF LDD, CHLORHEXIDINE AND TAP WATER.

Groups	Means	Variance	f-test	p-value	f-critical	Decision	Interpretation
tap water	0.3856	0.010954	246.0678	1.06E-16	2.866081	Reject Ho	There is significant Difference
CHLORHEXIDINE GRP.	1.3148	0.011611					
1:1 LDD GRP.	0.1862	0.001585					
1:10 LDD GRP.	0.1472	0.000719					
CONCENTRATED LDD GRP.	0.161	0.000435					

*LDD- liquid dishwashing detergent

Tissue repair is a complex physiological response which occurs after an injury. It is a multistep process that involves sequential phases, that is, hemostasis, inflammation, proliferation, and remodeling. Studies using tissue culture have revealed that tissues have an innate ability to reconstruct their structure (Chifflet & Hernandez 2016). Based on histopathological study, it showed that all groups undergone a natural healing process. The experimentation conducted is limited only for 16 days it includes the process of treatment and observation. Results such as reepithelization absent on surface

(groups I, III and V) and reepithelization incomplete for group IV signifies that the results requires more time for the tissue to be able to reach its full development of healing.

Figure 1: (section of group I : Tap water)

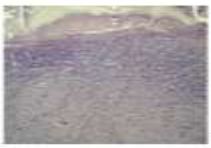
2) Histopathology report: scab and reepithelialisation incomplete or absent on surface; granulation tissue at the bottom of wound; neovascularisation, fibroplasias, with very minimal mononuclear inflammatory infiltrate noted.

3) histopathology report: scab and reepithelialisation absent on surface; granulation tissue formed at the bottom of wound; neovascularisation, fibroplasias, with moderate mononuclear infiltrate noted.

4) Histopathology result scab and reepithelization absent on surface; granulation tissue formed at the bottom of wound; neovascularization, fibroplasias, with marked mononuclear inflammatory infiltrate

Figure 2: (section of group II : Chlorheridine)

6) Histopathology result: thick scab composed of amorphous coagula covering the surfaceof wound. Beneath shows granulation tissue formation, neovascularization, fibroplasias with marked mononuclear infiltrate.



8) Histopathology study:): thick scab composed of amorphous coagula covering the surfaceof wound. Beneath shows granulation tissue formation, neovascularization, fibroplasias with marked mononuclear infiltrate

9) Histopathology study: scab and reepithelialization absent on surface; granulation tissue at the bottom of wound; neovascularisation, fibroplasias, with marked mononuclear inflammatory infiltrate noted.

Figure 3: (section of group III : DW 1:1)

11)Histopathology study: scab and reepithelialisation incomplete or absent on surface; granulation tissue at the bottom of wound; neovascularisation, fibroplasias, with marked mononuclear inflammatory infiltrate noted, with intralesional Langhan type giant cell (Pirogov-Langhans

12) Histopathology study:): thick scab composed of amorphous coagula covering the surfaceof wound. Beneath shows granulation tissue formation, neovascularization, fibroplasias with moderate mononuclear infiltrate

14) Histopathology result: scab and reepithelization absent on surface; granulation tissue formed at the bottom of wound; neovascularization, fibroplasias, with marked mononuclear inflammatory infiltrate

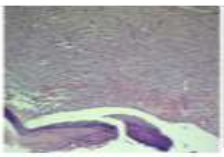
Figure 4: (section of group IV : DW 1:10)

16) Histopathology study: scab and reepithelialization absent on surface; granulation tissue at the bottom of wound; neovascularisation, fibroplasias, with marked mononuclear inflammatory infiltrate noted

17) Histopathology result: scab absent on surface; reepithelization incomplete, other areas with nude surface; granulation tissue at the bottom of wound; neovascularization, fibroplasia, with moderate mononuclear inflammatory infiltrate noted

18)) Histopathology study: scab and reepithelialization absent on surface; granulation tissue at the bottom of wound; neovascularisation, fibroplasias, with marked or severe mononuclear inflammatory infiltrate noted

Figure 5: (section of group V : DW pure)



21) Histopathology study: scab and reepithelialization incomplete, other areas withnude surface; granulation tissue at the bottom of wound; neovascularisation, fibroplasias, with moderate mononuclear inflammatory infiltrate noted.

23)) Histopathology study: scab and reepithelialization absent on surface; granulation tissue at the bottom of wound; neovascularisation, fibroplasias, with marked or severe mononuclear inflammatory infiltrate noted

24) Histopathology result): scab and reepithelization absent on surface; granulation tissue formed at the bottom of wound; neovascularization, fibroplasias, with moderate mononuclear inflammatory infiltrate noted

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In this study wound healing cannot be attributed to LDD alone. It is a multifactorial process that involves the immune system. Wound healing starts immediately after an injury. It involves three phases; inflammation, proliferation, and maturation. The 1st step is the inflammatory phase which involves platelet aggregation and infiltration of leukocytes such as neutrophils and macrophages into the site of injury. In the proliferative phase, re-epithelialization and formation of new granulation tissue begins. This action covers the wound area and is followed by tissue repair. Moreover, immunologic factors such as TGF-β1 promotes collagen deposition which is essential to tissue repair. TNF also promotes angiogenesis and collagen formation needed for tissue repair (Ishida et al., 2004).

IV. CONCLUSION

The ability of liquid dishwashing detergent in the inhibition of bacterial growth is an important factor in the process of wound healing as observed in the wound closure rate. The histopathological study supported the claim that LDD was able to promote wound healing, results such as neovascularization and fibroplasia as well as the presence of granulation signifies the presence of wound healing.

V. RECOMMENDATION

The researchers recommend the use of different concentration of liquid dishwashing detergent for further studies. In addition, different strain of bacteria which is common to wounds like E. coli ang P. aeruginosa should also be tested. Future researchers may also further study the specific content of dishwashing detergent that contributes to the wound healing effect, in this case the researchers recommend to conduct longer time frame of treatment and observation to meet the period of epithelialization.

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