THE DIFFERENCE OF EFFECTIVENESS BETWEEN CHITOSAN PLASTERS, NaCl, AND BETADINE IN HEALING WOUNDS ON GUINEA PIGS (Cavia Porcallus)

Rusmini[a], Andrayani
Nursing Department, Health Polytechnic of Ministry of Health in Mataram
[a] Corresponding author: rusmininr@gmail.com

ABSTRACT

Wounds are the breakdown or damage of tissue continuity, if not treated immediately there will be an infection or other complications so that it takes an action to treat wounds. Treatment of wounds can be done with ingredients that come from nature. This study aims to determine the effectiveness of shrimp shell extract plaster in the process of wound healing. This study was a pure experiment with a Post-test only control group design. Samples were 18 (6 for each group). The results showed that there were differences in the duration of wound healing between the three groups, statistical tests using one-way Annova Test and Post Hoc Test showed chitosan was more effective in wound drying than NaCl (p = 0.005 <0.05) and Betadine (p = 0,000 <0, 05). The wound closure process of chitosan group (8.6 days) was not significantly more effective than Na Cl (7 days) (p = 0.171> 0.05) and Betadine (10.8 days) (p = 100> 0.05), but the Na Cl group is faster than Betadine (p = 0.007 <0.05). Description of wound closure in chitosan is neater. The process of necrotic tissue detachment in the chitosan group (9 days) was no more effective than Na Cl (7 days) (p = 0.111> 0.05) and Betadine (10.8 days) (p = 142> 0.05). The release of necrotic tissue in the chitosan and NaCl groups did not leave a scar. Further research is recommended with other types of wounds and chitosan combinations with other natural antimicrobial agents.
Keywords: Wounds, Chitosan, Nacl, Betadine, Healing

INTRODUCTION

Wounds are the breakdown or damage of tissue continuity and if they are not treated immediately (poor treatment and handling) will lead to infection or other complications and leaving a permanent scar. The process of healing wounds needs treatment including wound cleansing and debridement, the applying of topical antibiotic preparat and bandaging (1). People generally used povidone iodine 10% or known as betadine to heal the wound, but the ingredients still have lack such as create scar tissue, irritation and also create resistance towards few types of microbe (2). Other than side effects that appear from povidone iodine 10% is irritation towards wound (3). This condition brought out other alternative wound treatment ingredient that is safe and have minimal side effect yet effective.

The development of further research suggests a wound treatment alternative that is much safer and along with the principle back to nature with herbal for honey, aloe vera, and gotu kola for burns shows that there is a speed-up in the wound healing process (4). Several kinds of research prove that there is an anti-bacterial process from the natural ingredients that gives positive effect in the wound healing process, namely from betel leaf extract, atung seed extract and Indonesian bay-leaf extract (5). One of the natural ingredients that proved to have an anti-bacterial characteristic is shrimp shell extract (5). In shrimp shell, there is a polisakarida known as chitosan that gives effect on anti-bacterial (6). The shrimp shell extract has chitosan that gives anti-microbial and anti-fungal effect (7) and also has a protective effect towards infectious agents (8).

Besides giving pharmaceutical effect, shrimp shell is a waste in the fishing industry that will give an adverse impact on the environment if it is neglected without proper handling. Therefore, shrimp shell needs to be used as it able to help the speed-up of wound healing while its use will lead to a reducing of environmental waste.

MATERIAL AND METHOD

The study employed true experimental design with post-test only control group design approach. The population of the study was guinea pigs (Cavia pocalllus) that taken from Pharmacology laboratory of Medical Faculty of University of Mataram. Sample of the study was 18 and then divided into 3 groups that consist of 6 samples for each group. The sampling technique used for each group was simple random sampling with inclusion and exclusion criteria. The inclusion criteria sample was *cavia porcallus,* between 2,5 until 3 months old, weight between 250-350 grams and in a healthy condition. The data was taken for 14 days on October 2016 with direct observation towards wound healing process using wound healing observation format consisted of observation of wound condition variables (the duration of wound drying), the duration of complete wound closure (the duration of healing) and the duration of necrotic tissue detachment. It

was later analyzed using Oneway Annova and continued by using Post Hoc test analysis.

RESULT AND DISCUSSION

The result shows that there is a difference between three groups namely three variables analyzed. Below is the table that explained the result of the study:

Table 1. The duration of wound drying

Variable	Group	Mean (day)	*One way Anova* Sig.	p-value	Post Hoc (LSD)		Sig.	p-value
Wound condition (Wound drying)	Chitosan	1	0,001	<0,05	Chitosan	NaCl	0,005	≤0,05
	NaCl	1,8				Betadine	0,000	<0,05
	Betadine	2,2			NaC	Chitosan	0,005	≤0,05
						Betadine	0,109	>0,05
					Betadine	Chitosan	0,000	<0,05
						NaCl	0,109	>0,19

Table 1 indicated that there is a difference of the duration wound drying between three groups where chitosan was more effective (1 day) compared to NaCl group (1,8 day) and Betadine (2,2 days). Statistic test using one-way ANOVA test and Post Hoc Test indicated that chitosan is more effective in wound drying than NaCl (p=0,005<0,05) and Betadine (p=0,000<0,05), but there is no difference between the duration of wound drying in NaCl group and Betadine (p=0,109>0,05).

Observation of wound condition was done macroscopically by looking at the condition of the wound's humidity. The result indicated that chitosan is more effective in wound drying. Shrimp shell extract (chitosan) is formed by monomer N-asetilglukosamin (GlcNAc) units that arranged linearly with β bounds (1,4). Chitin chains between one and another are associated with very strong hydrogen bonds between the N-H groups of one chain and C = O groups of adjacent chains. This hydrogen bound caused chitin able to form fibril formation (9) where it makes the shrimp shell extract (chitosan) can absorb fluids or exudates. This was supported by Hidayat (10) who proposed that wound dried faster in the group that applied with chitosan rather than the group that given NaCl in wound treatment.

Tabel 2. The duration of wound closure

Variable	Group	Mean (day)	*One way Anova* Sig.	p-value	Post Hoc (LSD)		Sig.	p-value
Wound condition (Wound closure)	Chitosan	8,6	0,024	<0,05	Chitosan	NaCl	0,171	>0,05
	NaCl	7				Betadine	0,100	>0,05
	Betadine	10,8			NaC	Chitosan	0,171	>0,05
						Betadine	0,007	<0,05
					Betadine	Chitosan	0.100	>0,05
						NaCl	0,007	<0,05

Table 2 presented the description of wound closure process at chitosan group (8.6 days) is not significantly more effective than NaCL (7 days) (p=0,171>0,05) and Betadine (19.8 days) (p=100>0,05), but the wound closure process for NaCl group is faster than Betadine group (p=0,007<0,05). The observation of wound closure duration was done macroscopically and entirely towards all wound condition until the wound was recovered marked by incision area has closed or blended perfectly.

The effectiveness of chitosan in wound closure was explained through the result of the study proposed by Pillai et.al (11) stated that chitosan could be used as fibroblast former applied as yarn, cover material and biodegradable substrates for the growth of human skin epithelium. Another research from Chiba *et al*. (12)

showed that animal testing that been given with chitosan has a proper neovascularization resolution, faster fibroblast induction, and more collagen fibers. This was supported by the research from Matsuoka et.a; (15) who stated that chitosan can improve half-time Basic Fibroblast Growth Factor compared to the control group by protecting it from being degraded by heat or enzymes that might damage it. *Basic Fibroblast Growth Factor* (bFGF) or known as FGF-2 is one of the prototypes of *Fibroblast Growth Factor* (FGF) that have an enormous effect towards the development of granulation tissue, a proliferation of fibroblasts, and angiogenesis, which these three factors have a function in wound closure process or wound healing.

Pavidone iodine has an anti-microbe effect that can create humid surrounding and able to induce angiogenesis. This drug has also been reported to prevent inflammation, but 10% of *Pavidone iodine* is also said to have an effect of inhibiting the growth of fibroblasts in vitro culture experiments (16). *Pavidone iodine* has benefit in accelerating the reepitelization and gives humid to the wound, but when it is compared to chitosan, chitosan able to stimulate the new tissue forming on the wound without making it humid on the gauze throughout the drying process.

The result also indicated that even though quantitatively the complete wound closure or the duration of wound healing between chitosan and betadine group does not show a significant difference statistically, but qualitatively there is a description indicated the difference between those two groups. The description of the wound closure on the chitosan group is neater and more symmetrical (edge of the wound is tidier), while the description of wound closure on the betadine group is untidy and look like burns. This is due to *Povidone iodine* that has lacks as it caused burns effects, pain, itchy and reddish and also lead to brownish scar (17)

One of the factors that influenced the wound healing process is the infection and the existence of pathogen microorganism into the wound (18) until it is needed to eliminate the infection to accelerate the healing of the wound. Related to this matter, either chitosan or betadine have anti-microbe effect until it can accelerate the wound healing. Betadine has a wide spectrum activity that able to kill vegetative bacteria, micro bacteria virus, and also fungus (19). Djamaluddin (20) explained in his study that chitosan accelerates the wound healing process as it can block the bacteria activity and eliminate the pus formation until the pus accumulation at the wound area could be avoided. It can be stated that various studies have proved the ability of chitosan as anti-microbe (5).

Few possibilities of chitosan as an anti-bacterial mechanism (5) is that chitosan is a polycationic that able to bind with negative charge from bacteria cell membrane through electrostatic interaction, until it influences the cell membrane permeability and lead to leakage of intracellular materials such as proteins, enzymes, genetic material, and others; therefore, chitosan as a metal chelating able to bind metal ions on intracellular liquid that have a crucial function for the continuity of bacteria cell; chitosan bind to the DNA and blocked mRNA and protein synthesis.

Table 3. The duration of necrotic tissue detachment

Variable	Group	Mean (day)	*One way Anova* Sig.	p-value	Post Hoc (LSD)		Sig.	p-value
The duration of necrotic tissue detachment	Chitosan NaCl Betadine	9 7 10,8	0,010	<0,05	Chitosan NaCl Betadine	NaCl Betadine Chitosan Betadine Chitosan NaCl	0,111 0,142 0,111 0,005 0,142 0,005	>0.05 >0,05 >0,05 ≤0,05 >0,05 ≤0,05

Table 3 indicated that the process of necrotic tissue detachment in chitosan group (9 days) was not more significantly effective compared to NaCl (7 days)

(p=0,111>0,05) and Betadine (10,8 days) (p=142>0,05). The necrotic tissue detachment on the chitosan group and NaCl do not leave a scar. The scar's description on betadine group is caused by the characteristics of betadine that can lead to burning effect (17), while chitosan and NaCl relatively safe for the tissue. According to clinical guidelines AHCPR 1994 in Setyawati et al (21), the solution recommended in cleaning and treating wounds is the normal saline solution as it is a physiological fluid and do not bring any harm to burn tissue. This is supported by Potter & Perry (22) stated that wound healing could be accelerated through wound treating using 0.9% NaCl solution as it is a physiological fluid and does not bring harm to wound tissue. Related to chitosan, Sezer (23), explained that a thinner epithelium in chitosan is better because in a thick epithelium still happen a fibroblast population stimulation process at the wound area and increasing some growth factor or mediator, therefore a perfect wound healing is a tissue with thin epithelium.

CONCLUSION

There was a difference between chitosan plaster, betadine, and NaCl in the wound healing process. In the wound drying process, the application of chitosan plaster is more effective compared to betadine and NaCl, but the chitosan wound closure process is not more effective compared to NaCl and betadine, but NaCl is more effective than betadine, but the description of wound closure on chitosan is neater. The process of the release of necrotic tissue on chitosan group (9 days) is significantly not more effective compared to NaCl and betadine but on NaCl chitosan do not have a scar after the necrotic tissue been released.

SUGGESTION

It is suggested that a further study is conducted with other kinds of wounds and chitosan combination with other anti-microbial agents.

ETHICAL CLEARANCE

The conducted study had been approved by the medical ethical commission of the University of Mataram No. 86/UN18.8/ETIK/2016 on July 30th, 2016. The research ethics has been considered by *beneficience dan non-beneficience* principle, justice for privacy and confidentiality.

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