ORIGINAL ARTICLE

Comparing Trigona, Apis dorsata Honey and Silver Sulfadiazine Effect on Bacteria Colonization in IIB burn

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Abstract

Objective: To compare the effect of Trigona honey, Apis dorsata honey and silver sulfadiazine against bacteria colonization in IIB burn on Rattus novergicus strain Wistar. **Design:** Design of this study is analytical experimental. This study used 27 rats randomly divided into 3 groups. All 3 group received deep second degree burns (2 x 2 cm).. The experimental group is divided into 2, in which 1 group receive 1 cc Trigona honey while the other receive 1 cc Apis dorsata honey, the control group is treated with silver sulfadiazine (SSD). Observation and data collection is done at fifth day. Bacteria colonization is obtained using quadrant streak method in blood agar media. The blood agar media is then incubated for 24 hours before being counted. **Results:** Statistical test using Mann- Whitney U found that Trigona honey and Apis dorsata honey yield equal in ability of suppressing bacteria colonization on grade IIB burn in rats. Trigona honey, Apis dorsata honey and SSD also yield equal ability in supressing bacteria colonization on grade IIB burn in rats. **Conclusion:** Trigona honey and Apis dorsata have equal ability in suppressing bacteria colonization on grade IIB burn in rats. They both also perform equal to SDD in suppressing bacteria colonization on grade IIB burn in rats.

Keywords: Burn; Honey; Trigona; Apis dorsata; bacteria colonization; SSD

Introduction

Burn is a form of tissue damages caused by heat, liquid, fire, steam, chemical substance, electricity, heat radiation and friction 1. Burn is classified by its depth into 3. Superficial burn (grade I), partial-thickness burn (grade II) which classified into 2 type; superficial partial-thickness (grade IIA) and deep partial thickness (grade IIB) and lastly full-thickness (grade III). Deep partial-thickness burn depth includes epidermis and some part of dermis 2.

Research that's conducted in Burn Unit GBPT (Gedung Bedah Pusat Terpadu) RSUD Dr. Soetomo Surabaya in January 1st – 31st December 2014 found there were 106 cases (48,4%) patient with grade IIB burn out of total 219 cases treated 3. Another research that's also conducted in Burn Unit GBPT RSUD Dr. Soetomo Surabaya in 2007-2011 discovered Pseudomonas is the most commonly found bacteria in burns, scoring at 20,7% 4.

Bacteria colonization generally doesn't interfere with the healing process however it can became dangerous if its lead to infection 5. Infection itself is bacteria invasion which colonize not only on the surface of the wound but but in addition to this inside and on the healthy tissues around the wound 6. One of the current therapy use to prevent infection in burn wound is silver sulfadiazine (SSD)

Recent studies found that SSD use can lengthen wound healing because SSD retard wound closure 7 and have toxic effect on keratinocytes 8. Honey as alternative medicine can be used to prevent infection without lengthening wound healing because it promotes autolytic debridement, stimulates growth of wound tissues and stimulates anti-inflammatory activities 9. Honey have antibacterial factors caused by its hyperosmotic state, low pH (3,2-4,5), it produces hydrogen peroxide and contain of Methylglyoxal (MGO) 9. Honey antibacterial activity can be classified into 2 groups, direct antibacterial activity and indirect antibacterial activity.

Direct antibacterial activity can be defined as honey ability in killing bacteria . Direct antibacterial activity include the formation of hydrogen peroxide (H2O2), high osmolality, low pH (3,2-4,5), non-peroxide factor and fenol. Indirect antibacterial activity can be defined as the host response to bacteria that is stimulated by honey. Indirect antibacterial activity include lymphocyte, antibody production, cytokines, immune response and nitrite oxide.

Hydrogen peroxide is formed slowly due to the interaction of wound exudate with the glucose oxidase that contained in honey. This formation happens optimally in aerobic situation. Study founds that more concentrated honey have lower concentration of hydrogen peroxide while honey with 30-50% in concentration have higher level of hydrogen peroxide. This data show that honey with concentration of 30-50% is the most suitable honey to use for wound dressing. Honey also contain ascorbic acid which help hydrogen peroxide in increasing lysis and bacteria death.

Hydrogen peroxide can be inactivated by heat, ultraviolet, MGO, catalase enzyme and auto-oxidation from flavonoid. Honey can still have antibacterial effect even though hydrogen peroxide is inactivated, this mechanism is called non-peroxide

antibacterial activity. Non-peroxide antibacterial activity is caused by peroxide acid and MGO. In Gram positive bacteria, MGO decrease the regulation of auto-lysine which involve in bacterial cell wall cleavage. In Gram negative bacteria, MGO organise gene expression which involve in bacterial cell wall stability.

This research is performed to differentiate the effect between Trigona honey, Apis dorsata honey and SSD on the amount of bacteria colonization Rattus novergicus strain Wistar with deep second degree on day 5 using microbiology examination. Day 5 is chosen to ensure bacteria colonization growth doesn't interfere with inflammation and proliferation.

Material and method

The design of this research is analytical experimental with posttest control group design. This study used twenty seven rats randomly divided into 3 groups; Trigona honey as P1 group, Apis dorsata honey as P2 group and silver sulfadiazine (SSD) as control group. An experimental skin burn model was prepared as described below. Rattus novergicus strain Wistar rats were anaesthetized with an intramuscular injection of ketamine (45 mg kg-1). The skin of dorsum was shaved. Burn injury was made as a contact burn with a square 2 x 2 cm metal plate which was previously immersed in boiling water 100o C for 5 minutes, onto the backs of the rats for 10 seconds. An hour after burn injury, each animal received an appropriate amount of topical formulation with regards to the assigned group. Group P1 received 1 cc of Trigona honey using disposable syringe, group P2 received 1 cc of Apis dorsata honey using disposable syringe and control group received 2 cm topical silver sulfadiazine (SSD) ointment topical. Transparent dressing is then given after burn wound is treated.

Data obtaining is performed at the same time with wound treating in fifth day. Data obtaining is carry out by swabbing the burn wound with sterile swab. Quadrant streaking technique is then used in blood agar media. After streaking, blood agar media is incubated in incubator for 24 hours.

Colony counting is done after the incubation was finished. Data obtaining was performed in Microbiology Laboratory Faculty of Medicine Universitas Airlangga Surabaya. The data was then classified into 4 groups, Rare, Few, Moderate and Abundant. Rare meaning the bacteria colony stops at the 1st quadrant, Few meaning the bacteria colony stops at the 2nd quadrant, Moderate meaning the bacteria colony stops at the 3rd quadrant and Abundant meaning the bacteria colony stops at the 4th quadrant.

Non parametrical statistic test is used to analyze the data because the bacteria colonization data denote scale of categorical data. Mann-Whitney U test is chosen to compare 2 group of independent sample. The first is to compare between P1 group and control group to examine the effect of Trigona honey. The second is to compare between P2 group and control group to examine the effect of Apis dorsata honey. Lastly to compare between P1 group and P2 group to differentiate the effect of Trigona honey and Apis dorsata honey.

Results

Table 1 shows control group, P1 group and P2 group all have the most bacteria colonization in moderate classification.

Table 2 shows that there's no significance amount of difference between P1 group and control group in bacteria colonization on rats with grade IIB burn (p-value = 0.377>∝). It can be said that Trigona honey and SSD have equal ability in supressing bacteria colonization on grade IIB burn in rats

Table 3 shows that there's no significance amount of difference between P2 group and control group in bacteria colonization on rats with grade IIB burn (p-value = 0.508>∝). It can be said that Apis dorsata honey and SSD have equal ability in supressing bacteria colonization on grade IIB burn in rats.

Table 4 shows that there's no significance amount of difference between P1 group and P2 group in bacteria colonization on rats with grade IIB burn (p-value = 0.693>∝). It can be said that Trigona honey and Apis dorsata honey have equal ability in supressing bacteria colonization on grade IIB burn in rats.

Discussion

There's no significant difference in bacteria colonization between Trigona honey and SSD. This can be due to direct antibacterial mechanism and indirect antibacterial mechanism. Direct antibacterial mechanism is due to hydrogen peroxide activity. While indirect antibacterial mechanism is due to non-peroxide activity. Non-peroxide activity is largely caused by flavonoids, low pH ± 3,3 and lysosome 10. Trigona honey peroxide activity is more dominant than its non- peroxide activity in aiding on its antibacterial activity 11

Research by Zainol et al 10 support researcher finding in which Trigona honey have antibacterial effect. This research used agar well diffusion method to determine its minimum inhibitory concentration (MIC) and its minimum bactericidal concentration on Trigona honey, Acacia honey, gelam honey, pineapple honey and tualang honey against the standard commercially available medical grade Manuka honey. It is found that Trigona honey have equal value of minimum inhibitory concentracion (MIC) and minimum bactericidal concentration (MBC). Bacteria used in this research is Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus cereus with Staphylococcus aureus as the most susceptible bacteria against Trigona honey because of its minimal MIC and MBC.

There's no significant difference in bacteria colonization between Apis dorsata honey and SSD. This can be due to low pH ± 3,8, invert sugar (mixture between glucose and fructose resulted from sucrose hydrolysis) and protein 12.

Research by Fahim et al 3 support researcher finding in which Apis dorsata honey have antibacterial effect. Method used is agar well diffusion in 20 different concentrations of honey ranging from 5% until 100%. This method is chosen to determine Apis dorsata honey inhibition zone. Bacteria used is Escherichia coli, Bacillus subtilis, Salmonella typhi, Pseudomonas aeruginosa and Aspergillus niger with Klebsiella pneumonia as the most susceptible bacteria because it have the biggest inhibition zone diameter.

Statistical analysis found that there is no significant difference in bacteria colonization between Trigona honey and Apis dorsata honey on rats with grade IIB burn. It can be said that Trigona honey and Apis dorsata honey have equal ability in supressing bacteria colonization.

This result is difference with the research conducted by V et al 9 that found there's a difference in antibacterial ability between Trigona honey and Apis dorsata honey. This difference is found by measuring Trigona honey and Apis dorsata honey inhibition zone against Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella enterica, Listeria monocytogenes, Escherichia coli dan Shigella flexneri bacteria. It is found that Trigona honey have bigger inhibition zone compared to Apis dorsata honey inhibition zone meaning Trigona honey have bigger antibacterial activity than Apis dorsata honey.

This research have its limitation such as this research doesn't have a baseline of bacteria colonization data (quantitative data), did not use negative control and the risk of contamination from environment can't be ruled out.

Conclusion

Trigona honey and Apis dorsata honey yield equal in its ability of suppressing bacteria colonization on grade IIB burn in rats, proven by its absence of difference in bacteria colonization between P1 group and P2 group. Trigona honey, Apis dorsata honey and SSD also yield equal ability in supressing bacteria colonization on grade IIB burn in rats.

Further research is required to find quantitative data such as using agar well diffusion method or dillution method, using control negative to ensure the difference in bacteria colonization and conducting research in a more sterile area to reduce the risk of contamination.

Conflicts of Interest

None

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Tables

Table 1 distribution of bacteria colonization

Bacterial Colonization	**GROUP**		
	CONTROL	**P1**	**P2**
Rare	1	0	0
Few	3	2	2
Moderate	4	6	7
Abundant	1	1	0
Sum (n)	9	9	9

Table 2 comparison result between P1 group and control group

GROUP	n	AVERAGE RANKING	TOTAL RANKING	*MANN-WHITNEY U*	*p-value*
P1	9	10.50	94.50	31.50	0.377
CONTROL	9	8.50	76.50		

Table 3 comparison result between P2 group and control group

GROUP	n	AVERAGE RANKING	TOTAL RANKING	*MANN-WHITNEY U*	*p-value*
P2	9	10.22	92.00	34.00	0.508
CONTROL	9	8.78	79.00		

Table 4 comparison result between P1 group and P2 group

GROUP	n	AVERAGE RANKING	TOTAL RANKING	*MANN-WHITNEY U*	*p-value*
P1	9	9.89	89.00	37.00	0.693
P2	9	9.11	82.00		