

BACTERICIDAL EFFECT OF TOPICAL *Vitis vinifera* TO METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) ON *Rattus norvegicus* SKIN WOUND

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ABSTRAK

Methicillin-Resistant Staphylococcus aureus (MRSA) merupakan salah satu patogen yang paling umum di seluruh dunia yang dapat menyebabkan berbagai infeksi kulit dan jaringan lunak dan bisa berakibat fatal menyebabkan sepsis. Pencarian obat dan kimia senyawa terhadap MRSA masih sangat diminati. Anggur (*Vitis Vinifera*) dikonsumsi secara luas di seluruh dunia, dan benihnya dianggap sebagai produk limbah, tetapi dalam penelitian *in vitro* terbukti menjadi sumber yang kaya senyawa polifenol yang menunjukkan efek antibakteri terhadap MRSA. Tujuan dari penelitian ini adalah untuk mengetahui efek antibakteri dari ekstrak biji anggur pada MRSA pada luka kulit pada tikus putih (*Rattus norvegicus*) strain Wistar dan membandingkan efek antibakteri mupirocin pada MRSA. Desain penelitian berupa eksperimen menggunakan *posttest only controls group design*. Luka di bagian belakang 27 tikus putih galur Wistar jantan (*Rattus norvegicus*) diinokulasi dengan MRSA. Spesimen diambil 6 jam setelah inokulasi MRSA, pada hari 1 dan hari ke-3 setelah pengobatan dengan ekstrak biji anggur (*Vitis vinifera*) dan mupirocin untuk pemeriksaan mikrobiologi menghitung jumlah koloni. Pada semua luka yang diterapkan ekstrak biji anggur, jumlah koloni MRSA secara signifikan meningkat sehubungan dengan kontrol dalam 1 x 24 jam ($p < 0,005$) dan penurunan ($p < 0,0001$) setelah 3 x 24 jam, sedangkan yang diobati dengan mupirocin menurun secara signifikan dalam waktu 1 x 24 jam. Ekstrak biji anggur memiliki efek antibakteri, tetapi memiliki efek yang lebih lambat dibandingkan dengan mupirocin. (FMI 2015;51:13-15)

Kata kunci: MRSA, efek antibakteri, *Vitis vinifera*, polifenol

ABSTRACT

Methicillin Resistant Staphylococcus aureus (MRSA) is one of the most common pathogens worldwide that can cause variety of skin and soft tissue infection and can be fatal causing septicemia. Searching for drug and chemical compounds against MRSA is still in demand. Grape (*Vitis Vinifera*) is consumed widely around the world, and the seed is considered as a waste product but study *in vitro* proven to be a rich source of polyphenolic compounds that show antibacterial effect against MRSA. The aim of this study is to investigate the antibacterial effect of grape seed extract on MRSA on white rat (*Rattus norvegicus*) Wistar strain skin wound and compare the antibacterial effect to mupirocin's effect on MRSA. Experimental research design using *posttest only controls group design*. The wound at the back site of 27 healthy male white rats Wistar strain (*Rattus norvegicus*) inoculated with MRSA. The swab specimens taken 6 hours after inoculation of MRSA, on day 1 and day 3 after the treatment with grape seed extract (*Vitis vinifera*) and mupirocin for microbiological examination to count the number of colonies. On all of the wounds in which were applied topical grape seed extract, the number of colonies of MRSA was significantly raised with respect to the control in 1 x 24 hours ($p < 0,005$) and decreased ($p < 0,0001$) after 3 x 24 hours, while treated with mupirocin significantly decreased within 1 x 24 hours. Grape seed extract has antibacterial effect, but it has slower effect compared to mupirocin. (FMI 2015;51:13-15)

Keywords: MRSA, antibacterial effect, *Vitis vinifera*, polyphenolic

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INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to many antibiotics (Graham et al 2006). MRSA was first reported case occurred in 1961 at a hospital in England, while in the hospital in the United States first in 1968. MRSA is a bacterium that most commonly found as pathogens worldwide. In the United States, the incidence of MRSA infections are increasing, being found 2.4% of nosocomial infections in the late 1970s to

29% in 1991, and 43% in 2002 (Duquette & Nuttall 2004, CDC 2008). MRSA can cause a variety of skin and soft tissue infections including impetigo ulcers, folliculitis, furunculosis, cellulitis, and abscess. This strain is a major cause of nosocomial infections associated with medical devices and surgical site infection. MRSA threat after entering the bloodstream causes pneumonia, endocarditis, septic arthritis, osteomyelitis, meningitis and septicemia (Alex & Letizia 2007). Infection with multidrug-resistant MRSA will extend the length of hospitalization, increased cost

of care, leading to additional morbidity and even death (Nathwani 2003).

Grape (*Vitis vinifera*) is consumed widely around the world, and is known to contain many phenol components. Approximately 60-70% of the total phenol component is in the grape seeds. Phenolic are known to have therapeutic activities, acting as immunomodulators, antioxidants, antimutagens, antibacterials and hormone analogs (Halpern et al 1998). Grape seed extract contains many polyphenolic antioxidants that have the potential as an antibacterial agent thus expected to inhibit the growth of MRSA. The in vitro study measured antibacterial activity of grape seed extract against all strains of MRSA showed MRSA sensitive to grape seed extract and complete inhibition occurred at concentrations of grape seed 3mg/ml proanthocyanidin extract. Antibacterial activity was bactericidal as shown by disruption of the bacteria cell wall (Al-Habib et al 2010). That study became the basis for doing further research in vivo, to see the antibacterial effect of grape seed extract against MRSA on skin lesions of rat and compared its effect with mupirocin (Bactroban®) which is known as a topical antibiotic option to inhibit the growth of MRSA (Laupland & Conly 2003) and the purpose of this study was to prove a topical antibacterial effect of grape seed extract against MRSA on rat skin wound.

MATERIALS AND METHODS

This study was an experimental research design using posttest only control group design. The sample size used was determined by the Federer Formula, 9 rats for each group. (9 untreated rats as control, 9 rats treated with grape seed extract, 9 rats treated with mupirocin). Selected 27 healthy male rats Wistar strain (*Rattus norvegicus*) about 3 months old, weighting 200-250 g. The rats were maintained in cages and given food and drink with the same number and type. Wound sites were prepared on the back of each anesthetized rat by exposing 2 cm² of fascia (Rats anesthetized with ketamine 20 mg/kg body weight intramuscularly). Each wound was inoculated by MRSA and then covered with a transparent dressing to prevent contamination to the surrounding area.

The grape purchased from the local market and then their seed were removed, washed with distilled water, dried under shade 25°C till constant weight and powdered with blender. One hundred fifty gr powdered extracted with alcohol for 24 hours, the defatted material filtered and concentrated under vacuum to get crude extract. The extracts were concentrated to dryness under reduced pressure and controlled temperature (60°

C) in a rotary evaporator. Mupirocin, used were mupirocin calcium or equal to 2% pure mupirocin (Bactroban®), applied topically on the rat skin wound. The initial specimen were taken before 6 hours post inoculation of MRSA and considered as control and then treat the wound with grape seed extract or mupirocin. The next specimen was taken on day 1 and day 3 after the treatment. The specimen taken will then be inserted into the culture swab tube obtained from Microbiology Laboratory Dr. Soetomo Hospital Surabaya. Specimens sent to the microbiology laboratory for microbiological examination of the bacterial colonization of MRSA.

RESULT

In less than 6 hours after the rats were wounded and inoculated with MRSA, the culture results from white rats skin wounds obtained a significant number of MRSA colonies with $p < 0.05$ ($p = 0.01$) both in the control group, the treatment group by administering grape seed and the group treated with mupirocin addition, where in each group got a different number of MRSA colonies as shown in table 1.

Table 1. MRSA Cultures in all groups in less than 6 hours after MRSA inoculation

	Control	Grape seed extract	Mupirocin	P
Mean	10.7 ^a	92.4 ^b	118.8 ^b	0.010
SD	11.8	76.1	106.7	
Minimum	0	20	1	
Maximum	38	256	331	

After 1 x 24 hours in the control group and group with grape seed extract found an increasing number of MRSA colonies, whereas in the group given mupirocin the number of MRSA colonies decreased. And in 3 x 24 hours the number of MRSA colonies in the control group remained elevated, whereas in the group with grape seed extract and mupirocin decreased (Table 2).

Table 2. Mean MRSA colony in the culture

Day	Control	Grape seed Extract	Mupirocin
0	10.7	92.4	118.6
1	95.7	16061	25.4
3	208.6	87.3	10.7

DISCUSSION

Grape seed extract contains many polyphenols as potential antibacterial, that effect is thus expected to inhibit the growth of MRSA. Statistical analysis showed that at the end of the study, it proved that grape seed extract can inhibit the growth of MRSA, so it has bactericidal effect. Several in vitro studies proved that grape seed extract could inhibit the growth of MRSA (Al-Habib et al 2010, Jayaprakasha et al 2003). This research has been proven the effect in vivo and evaluated changes in the number of MRSA colonies after addition of grape seed extract on rats skin wounds that were previously inoculated with MRSA. Furthermore this study also compared the antibacterial effects of grape seed extract with mupirocin.

Bagchi et al. studied an acute dermal toxicity of grape seed extract on albino rats intact skin, and it was found that grape seed extract could cause slight erythema and desquamation that could subsided in less than 12 days. And they found LD50 of grape seed extract was greater than 2000 mg/kg body weight, when administered once for 24 hours (Bagchi et al 2000). In this study the results of statistical analysis in 1 x 24 hours after administration of grape seed extract revealed that MRSA colonies were greatly increased compared with controls group. It might be caused by the dermal irritation that made the skin barrier mechanism declined and furthermore increased MRSA colonies, but then 2 days later the number of MRSA colonies when compared with less than 6 hours after the wound was inoculated with MRSA is decrease significantly ($p = 0.0001$).

CONCLUSION

Topical grape seed extract has bactericidal effect against MRSA. Compared to mupirocin, it showed that the effect seemed slower at 1 x 24 hours after the addition, but achieved the similar bactericidal effect after 3 x 24 hours.

REFERENCES

- Alex A and Letizia M (2007). Community-acquired methicillin-resistant *Staphylococcus aureus*: considerations for school nurses. *J Sch Nurs* 23, 210-213
- Al-Habib A, Al-Saleh E, Safer AM, Afzal M (2010). Bactericidal effect of grape seed extract on methicillin-resistant *Staphylococcus aureus* (MRSA). *J Toxicol Sci* 35, 357-364
- Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS, Pruess HG (2000). Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology* 148, 187-197
- CDC (2008). About Methicillin-Resistant *Staphylococcus aureus* (MRSA) Among Athletes. Available from http://www.cdc.gov/ncidod/dhqp/ar_MRSA_AthletesFAQ.html. Accessed March 9, 2012
- Duquette RA and Nuttall TJ (2004). Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? *J Small Anim Pract* 45, 591-597
- Graham PL 3rd, Lin SX, Larson EL (2006). A US population-based survey of *Staphylococcus aureus* colonization. *Ann Intern Med* 144, 318-325
- Halpern MJ, Dahlgren AL, Laakso I, Seppänen-Laakso T, Dahlgren J, McAnulty PA (1998). Red-wine polyphenols and inhibition of platelet aggregation: possible mechanisms, and potential use in health promotion and disease prevention. *J Int Med Res* 26, 171-180
- Jayaprakasha GK, Selvi T, Sakariah KK (2003). Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Research International* 36, 117-122
- Laupland KB and Conly JM (2003). Treatment of *Staphylococcus aureus* colonization and prophylaxis for infection with topical intranasal mupirocin: an evidence-based review. *Clin Infect Dis* 37, 933-938
- Nathwani D (2003). Impact of methicillin-resistant *Staphylococcus aureus* infections on key health economic outcomes: does reducing the length of hospital stay matter? *J Antimicrob Chemother* 51, ii37-ii44