



Comparison Of The Effectiveness Of Aloe Vera Extracts and Silver Sulfadiazine on Malondialdehyde and Superoxide Dismutase Levels With Biomarker C-Reactive Protein In Rats With Mid Dermal Burns

Eurika Lawrence¹, Gusbakti Rusip², Erny Tandanu³, Ade Indra Mukti⁴, Andriady Saidi Nasution⁵, Anita Rosari⁶, Yunita Dewani⁷

¹Post Graduate, Faculty of Medicine University Prima of Indonesia, ²Department of Exercise Physiology, Faculty of Medicine University Prima of Indonesia, ³Department of Pharmacology Therapeutic, Faculty of Medicine University Prima of Indonesia, ⁴Department of Urology, Faculty of Medicine University Prima of Indonesia, ⁵Department of Urology, Faculty of Medicine University Prima of Indonesia, ⁶Department of Internal Medicine, Faculty of Medicine University Prima of Indonesia, ⁷Department of Anesthesiology, Faculty of Medicine University Prima of Indonesia.

Accepted set by Gusbakti Rusip.

Correspondent author: Gusbakti Rusip

*Correspondent email : gusrusip@gmail.com

ABSTRACT

Background : Burns caused by radiation, heat, chemicals, electricity or friction can cause damage to tissues including the skin. Aloe vera (Liliaceae family) is one of the most recognized herbs in the world. Glucomannan and acemannan in aloe vera are the active chemical compounds which can stimulate proliferation and fibroblast activity. Meanwhile, Silver sulfadiazine (SSD) is the gold standard in burn therapy because it is a broad spectrum antibiotic and is not resistant. However, in several studies on SSD it was found that SSD can inhibit wound healing in vivo, cytotoxic effect on keratinocytes and fibroblast in vitro. After burn injury, ROS is being produce in a large amount and can be dangerous. ROS also has implication for tissue damage, infection and sepsis, inflammation, systemic response syndrome, multiple organ failure and immunosuppression. Free radicals change the components of cell membranes and produce toxic compounds such as MDA (Malondialdehyde) which are formed as a result of lipid peroxidation. Superoxide Dismutase enzyme is an enzyme that plays a role in the first process to counteract free radicals. An increase in the inflammatory response also occurs in burns causing an increase in CReactive Protein. This aim of this study is to determine the effectiveness of Aloe Vera extract and SSD on MDA and SOD levels with CRP as a biomarker in rats with Mid-Dermal burns.

Research method: This study examined 25 male wistar rats treated with Mid Dermal burns. rats were then divided into 5 groups: normal, negative control, the burn group treated with Aloe Vera extract, SSD, and combination of Aloe Vera extract with SSD for 14 days. MDA and SOD measurements were made after 14 days. CRP measurements were carried out in rats on days 3 and 15 randomly.

Results: It is shown that the group receiving Aloe Vera extract / SSD / combination of Aloe Vera extract and SSD can reduce MDA levels and increase SOD levels ($p < 0.05$), but the group receiving Aloe Vera extract and SSD combination therapy is more effective in reducing MDA levels and increased SOD levels compared to the group with single therapy Aloe Vera extract / SSD. CRP levels were also seen to be lower in the group of rats that received combination therapy with Aloe Vera extract and SSD.

Conclusion: Administration of a combination of Aloe Vera extract and SSD for 14 days was more effective in increasing SOD levels and reducing plasma MDA levels as indicated by improvements in burns and evidenced by lower CRP levels compared to other groups where CRP is a biomarker for the degree of infection.

Keywords: Aloe Vera Extract, Silver Sulfadiazine, mid dermal burn, MDA, SOD, CRP.

INTRODUCTION

Burns caused by radiation, heat, chemicals, electricity or friction can cause damage to tissues including the skin. (American Burn Association, 2019). Multiple mechanisms are involved in the development of burns, including micro thrombosis, hypoperfusion, vasoconstriction, and oxygen free radical damage, which can lead to a cascade of premature inflammation and necrosis or apoptosis (cell death). Various mediator like cytokines, inflammatory mediators and reactive oxygen species (ROS) are being release after burns. (George, Suchithra, Bhatia, 2021)(Zhou et al., 2021). After burn injury, ROS is being produce in a large amount and can be dangerous. ROS also has implication for tissue damage, infection and sepsis, inflammation, systemic response syndrome, multiple organ failure and immunosuppression.

Free radicals change the components of cell membranes and produce toxic compounds such as MDA (Malondialdehyde) which are formed as a result of lipid peroxidation. The higher the MDA level, the more oxidative damage that occurs and has a negative impact on health. (Dunnil et al, 2017)(Herdiani, Putri, 2018) The body has the ability to form endogenous antioxidants that aim to counteract free radicals, for example GPx (Glutation Peroxidase), catalase and Superoxide Dismutase (SOD) enzyme activity. The first enzyme that counteract free radicals is SOD. Metal minerals such as manganese (Mn), zinc (Zn), and copper (Cu) play an important role in helping SOD enzyme activity to work optimally. (Yulianti, 2015)

Severe burns can cause high risk of infection and inflammation. C-reactive protein or CRP is a common marker of acute inflammatory response and increasing in CRP levels are related with the clinical outcome of infection. (Putra et al., 2020)

Silver sulfadiazine (SSD) is the gold standard in burn therapy because it is a broad spectrum antibiotic and is not resistant. However, in several studies on SSD it was found that SSD

can inhibit wound healing in vivo, cytotoxic effect on keratinocytes and fibroblast in vitro. Several side effects and adverse reactions have been reported after using SSD on a large wound and for a long time, such as leukopenia, resistance to SSD, and renal toxicity (Ihsan, Rahmadian, Raymond 2021)

Traditional medicine such as Aloe vera (Liliaceae family) has long been widely used in community. According to the study of Hosseinimehr, et al. demonstrated aloe vera more effective in healing partial burns in rats compare to SSD. Glucomannan and acemannan in aloe vera are the active chemical compounds which can stimulate proliferation and fibroblast activity. (Aulia, Pane, 2022).

This research aim to determine the effectiveness of Aloe Vera extract and SSD on MDA and SOD levels with CRP as a biomarker in rats with MidDermal burns.

The benefit of this research is as a source of information regarding the effectiveness of using aloe vera and SSD in rats with Mid Dermal burns.

METHODS

This is an experimental study in an animal burnmodel with a post test only group design.

1. ETHICS

This research was approved by the Ethics Clearance Committee of the Faculty of Medicine, Universitas Prima Indonesia.

2. Experimental Animal Twenty five male wistar rats were managed in the Pharmacology Laboratory, Faculty of Pharmacy, University of North Sumatra, Medan, Indonesia. Body mass between 200-300g. Before starting the experiment, the animals were adapted for 1 week.

3. Allocation and treatment groups The sample in this study was 25 rats. Those rats divided in 5 Groups which is one group contain 5 samples. First group was the normal control group, second group received wound care using 0.9% NaCl, group 3 received wound care using Aloe Vera extract twice a day, group 4 received

wound care using SSD twice a day and group 5 received wound care using a combination of Aloe Vera extract and SSD for twice a day.

4. Wound Infliction

All samples will be given anesthesia before performing the burn with Ketamine by intraperitoneal injection applied to the rats in order to ensure that the rats remain in comfort feel and pain free. All rats sample will be hairfree at dorsal part before the burn apply. Iron plate measured in 2×2 centimetres square size will be use to inflict the mid dermal burn wound. The iron plate heated in boiling water for 5 minutes and apply the hot surface for 30 seconds on the shaved dorsal area.

(Kristyaningsih, 2016).

5. Aloe Vera Extract

To produce aloe vera extract, 2000grams fresh aloe vera leaf collected. To clean out the skin, get rid the spiky edges on the top and alongside the plant and slice off the skin on the flat bottom side. Approximately 1000g of aloe gel weighed and washed thoroughly and divided it by cutting in small cube size. Add 10 liters of 70% ethanols, stirred periodically anytime for 6 hours, then cooling it down for another 18 hours. Then, the small pieces of gel are blended and poured into a sealed container. First filtrate from the mixture was filtered to obtain filtrate. Second filtrate process is another extraction on the residue follow by using 5 litres of ethanol contains 70% Alcohol to obtain another form filtrate. First and second filtrate well mixed and evaporated by using tool vacuum rotary evaporator (Heidolph Instruments GmbH & Co. KG, Germany) at 45°C temperature to obtain a semi solid extract.

6. Gel Preparation

- Gel Base

Weigh 0.5 grams of carbopol, 1 gram of tea and then expand the carbopol for 24 hours, weigh 5 grams of propylene glycol, 0.1 nipagin and 0.02 grams of nipasol. After 24 hours, grind the carbopol that has grown earlier then add the tea until a gel

base is formed, then dissolve the nipagin and nipasol in hot water, then mix them into the gel base.

- Aloe vera gel
40 grams of aloe vera extract, blend with 360 gram of gel base, grinding all by using mortar until homogeneous.

7. Sample collection

Blood sampling was carried out from the orbital sinus, provided that previously the test animals were fasted for 16 hours. Blood was taken on the 3rd and 15th day, by collecting 3 milliliters of hematocrit in an Eppendorf tube containing EDTA. Centrifugation was then carried out on blood at a rate of 1000 rpm at 4°C for 10 minutes, plasma was formed which was then transferred and allowed to stand at -80°C. The buffer solution formed was removed from the erythrocyte pellet, then the erythrocytes were dissolved 5X in volume using ddH₂O.

Centrifugation was carried out again at a rate of 10000 rpm for 10 minutes, then a supernatant was formed which was then allowed to stand at 80°C

8. Analysis of Malondialdehyde, Superoxide Dismutase, C-Reactive Protein

Concentrations of MDA, SOD and CRP levels were determined using a blood spectrophotometer at a wavelength of 545 nm, 550 nm and 450 nm.

9. Statistical analysis

Statistical Program Service Solution (SPSS) for Windows is used to process and analyze data. This study uses the One Way Anova test because the data is normally distributed (Shapiro-Wilk test) and homogeneous (Levine test). Then proceed with the LSD test if there is a significant difference between groups. Statistical test was carried out at 95% confidence level ($p < 0.05$)

RESULT

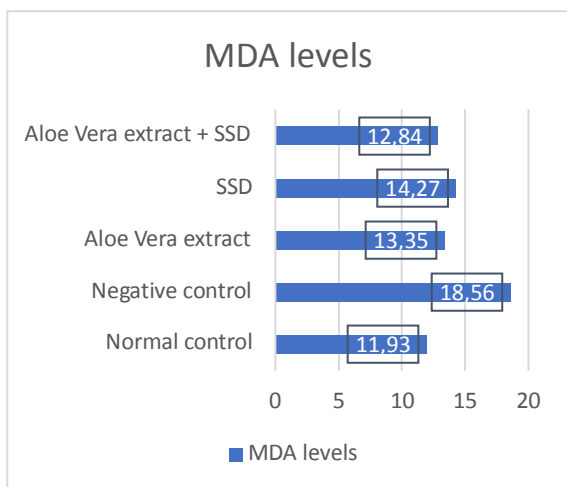


Figure 1. Statistic number analyzed by ANOVA show significant difference on mean number of Malondialdehyde in rats base from treatment *p<0.05

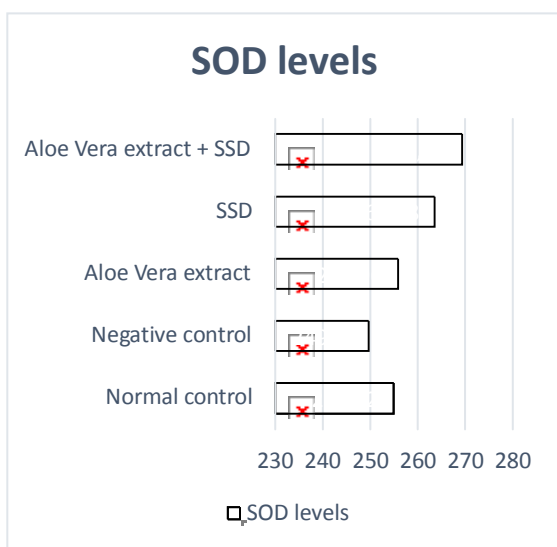


Figure 2. Statistic number analyzed by ANOVA show significant difference on mean number of SOD levels in rats base from treatment *p<0.05

SOD and MDA levels in blood plasma in each treated group and given Aloe Vera extract / SSD / a combination of Aloe Vera extract and SSD showed a significant difference between groups (p <0.05). The highest levels of MDA

were seen in negative control. Meanwhile, the highest levels of SOD were seen in combination of Aloe Vera extract + SSD therapy.

p	Group II	Sig.
Normal Control	Negative control	0.070
	Aloe Vera Extract	0.000
	SSD	0.000
Aloe Vera Extract	Aloe Vera Extract + SSD	0.010
	Normal Control	0.000
	Aloe Vera Extract	0.000
SSD	SSD	0.000
	Aloe Vera Extract + SSD	0.000
	Normal Control	0.000
Aloe Vera Extract + SSD	Normal Control	0.000
	Negative Control	0.000
	SSD	0.009
Negative Control	Aloe Vera Extract + SSD	0.120
	Normal control	0.000
	Negative control	0.000
Aloe Vera Extract + SSD	Aloe Vera Extract	0.009
	Aloe Vera Extract + SSD	0.000
	Normal Control	0.010
Normal Control	Negative Control	0.000
	Aloe Vera Extract	0.120
	SSD	0.000

Table 1. Post hoc test results comparing Aloe Vera extract and SSD to MDA levels in rats with Mid-Dermal burns.

Group I	Group II	Sig.
Normal Control	Negative control	0.180
	Aloe Vera Extract	0.035
	SSD	0.805
Aloe Vera Extract	Aloe Vera Extract + SSD	0.001
	Normal Control	0.180
	Aloe Vera Extract	0.002
SSD	SSD	0.116
	Aloe Vera Extract + SSD	0.000
	Normal Control	0.035
Aloe Vera Extract + SSD	Negative Control	0.002

	<u>SSD</u>	<u>0.157</u>
	Aloe Vera Extract + SSD	0.138
SSD	<u>Normal control</u>	<u>0.805</u>
	<u>Negative control</u>	<u>0.116</u>
	<u>Aloe Vera Extract</u>	<u>0.057</u>
	Aloe Vera Extract + SSD	0.002
Aloe Vera Extract + SSD	<u>Normal Control</u>	<u>0.001</u>
	<u>Negative Control</u>	<u>0.000</u>
	<u>Aloe Vera Extract</u>	<u>0.138</u>
	<u>SSD</u>	<u>0.002</u>

Table 2. Post hoc test results comparing Aloe Vera extract and SSD to SOD levels in rats with Mid-Dermal burns.

From table 1, there was a significant difference ($p < 0.05$) in MDA levels for almost all groups except for Aloe Vera extract with SSD, Aloe Vera extract with Aloe Vera extract and SSD. While table 2 showed significant differences in SOD levels ($p < 0.05$) in the normal control group with Aloe Vera extract, normal control with Aloe Vera extract and SSD, negative control with Aloe Vera, negative control with Aloe Vera extract and SSD, as well as the SSD group with Aloe Vera extract and SSD.

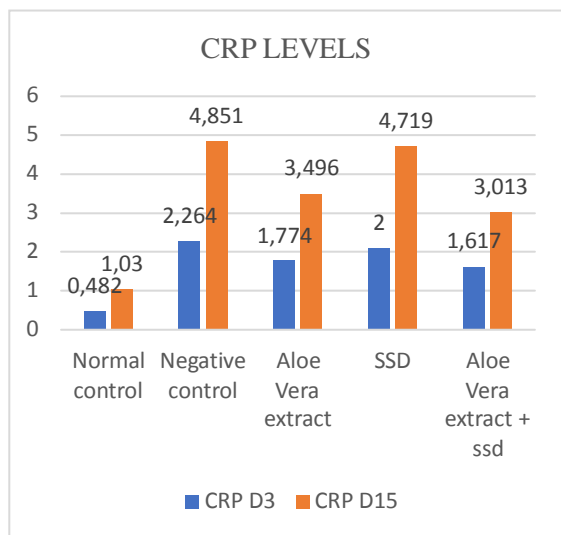


Figure 3. Statistic number analyzed by ANOVA show significant difference on mean number of C-Reactive Protein in rats base from treatment

$*p < 0.05$	
Treatment	P-value
Normal control	0.070
Negative control	0.009
Aloe Vera extract	0.026
SSD	0.002

Aloe Vera extract + SSD 0.001 Table 3. Paired T Test CRP results in rats with Mid Dermal burns.

Based on the table above (table 3), it can be seen that $p < 0.05$ in the Paired T-Test showed that there was a significant difference in CRP on days 3 and 15 in all group that receives burn treatment.

DISCUSSION

In this study, MDA levels in blood plasma of the group of rats induced by burns and given Aloe Vera extract / SSD / a combination of Aloe Vera extract and SSD were lower and SOD was higher when compared to the group of rats which induced by burns (negative control) without administration of Aloe Vera extract / SSD / combination of Aloe Vera and SSD extracts. This shows that both Aloe Vera extract and SSD can decreasing MDA levels and elevating SOD levels in the blood plasma of the group of rats induced by Mid Dermal burns. Oxidative stress is a reaction that occurs when ROS levels increase and exceeds the endogenous defense capacity. Oxidative stress causes disruption of proteins and nucleic acids, lipid peroxidation of cell membranes, which contribute to progressive tissue damage in the burn stasis zone, cell death and decreased SOD activity. (Gusbakti et al., 2022) Increased levels of MDA and decreased levels of SOD indicate the occurrence of immensely lipid peroxidation processes in cells and as a sign of oxidative stress in the body. MDA which is the final process of lipid peroxidation will be measured to measure the degree of oxidative stress in the body. (Moselhy et al., 2013). In this study, blood plasma MDA levels of the negative control group that were given burns increased and SOD levels decreased compared to the normal group that were not given burn treatment. This result is in line with

previous research that burns will produce excess Reactive Oxygen Species (ROS) thereby reducing antioxidant defenses in the body and the result is oxidative stress and triggering a chain reaction that damages cells and causes wound healing to fail. (Zhang, 2020).

Maenthaesong et al, in their systematic review results showed that the content of Aloe Vera in various preparations effectively succeeded in accelerating the process of wound healing and always increased the success rate of wound healing and the rate of epithelialization of first and second degree burns when compared to healing with conventional treatment methods. This is in line with this study where Aloe Vera extract was proven to reduce ROS and was proven by a lowering of MDA levels and an elevation of SOD levels.

Severe burns also can cause high risk of infection and inflammation. Acute inflammatory response common marker is C-Reactive Protein,

Increasing in CRP levels linked with clinical outcome of the infection. (Putra et al., 2020) Aloe Vera is a popular plant that is widely used as a traditional medicine for healing burns, has antiseptic, anti-inflammatory, immunostimulant, and anti-tumor. Aloe Vera is one of several ingredients known to be effective at reducing inflammation.

According to Muller et al, study showed that SSD delayed wound contraction in burn injury. SSD when added to Aloe vera reversed this effect and wound half-life, and the healing time was longest in the 1% SSD group and the shortest in the SSD/Aloe vera group. These data indicate compared to SSD alone, Aloe vera has a synergistic effect in wound healing.

This is proven in this study by CRP examination in each group, where three rats in each group were randomly examined for CRP levels on days 3 and 15 and found that CRP levels in the combination therapy of Aloe Vera and SSD was the lowest (figure 3).

CONCLUSION

- a. Aloe vera extract has been shown to increase SOD levels and reduce plasma MDA levels in rats with Mid-Dermal burns.
- b. SSD has been shown to increase SOD levels and reduce plasma MDA levels in rats with Mid-Dermal burns.
- c. Compared with SSD, Aloe Vera extract was more effective in increasing SOD levels and reducing blood plasma MDA levels in rats with Mid-Dermal burns.
- d. Administration of a combination of Aloe Vera extract and SSD for 14 days was more effective in increasing SOD levels and reducing plasma MDA levels as indicated by improvements in burns and evidenced by lower CRP levels compared to other groups where CRP is a biomarker for the degree of infection.

REFERENCES

1. ABA: 2018. *Advanced Burn Life Support Course Provider Manual*. American Burn Association.
2. ABA: 2019. *Advanced Burn Life Support Course Provider Manual*. American Burn Association.
3. Adhya,A., Bain,J., Ray, O., Hazra, A., Adhikari, S., Dutta, G., Ray, S., Majumdar, B, K., 2015. Healing of burn wounds by topical treatment: A randomized controlled comparison between silver sulfadiazine and nano-crystalline silver.
4. Aulia ,L., Pane ,Y, S., 2022. Effect of Aloe vera extract in post-burn skin repair in rats.
5. Bunyapraphatsara ,N., Jirakulchaiwong, S., Thirawarapan, S., Manokul, J., 1996. The Efficacy of Aloe Vera Cream In The Treatment of First, Second, and Third Degree Burns in Mice.
6. Dana ,N ., Javanmard, S, H., Asgary, S., Asnaashari, H., Abdian, N., 2022. The Effect of Aloe Vera Leaf Gel On Fatty Streak Formation In Hypercholesterolemic Rabbits.

7. Duansak ,D., Soomboonwong, J., Patumraj ,S., 2003. Effects of Aloe Vera on Leukocyte Adhesion and TNF-Alpha and IL-6 Levels in BurnWounded Rats.
8. Dunnill ,C., Patton ,T., Brennan, J., Barrett, J., Dryden, M., Cooke, J., Leaper, D., Georgopoulos, N, T., 2015. Reactive oxygen species (ROS) and wound healing:the functional role of ROS and emerging ROS- modulating technologies for augmentation of the healing process.
9. Fenny., 2022. Terapi Medik Gizi Pada Pasien Luka Bakar Berat: Peran Pemberian Vitamin D.
10. George, B., Suchithra ,T, V., Bhatia ,N., 2021. Burn injury induces elevated inflammatory traffic: the role of NF-kB.
11. Georgiade S. G., Christopher W. P., 2011. Buku Ajar Bedah . edisi:17. Jakarta, Indonesia: Penerbit Buku Kedokteran EGC.
12. Harun, I., Susanto, H., Rosidi, A., 2017. Pemberian tempe menurunkan kadar Malondialdehyde (MDA) dan meningkatkan aktivitas enzim Superoxide Dismutase (SOD) pada tikus dengan aktivitas fisik tinggi.
13. Herdiani, N., Putri , E, B, P., 2018. Pengaruh ekstrak buah naga terhadap kadar MDA tikus yang diberi paparan asap rokok.
14. Horton , J , W., 2003. Free Radicals And Lipid Peroxidation Mediated Injury In Burn Trauma: The Role of Antioxidant Therapy.
15. Hosseinimehr ,S , J., Khorasani ,G., Azadbakht, M., Zamani, P., Ghasemi, M., Ahmadi, A., 2010. Effect of Aloe Cream versus Silver Sulfadiazine for Healing Burn Wounds in Rats.
16. Hussain , Z., Thu, H.E., Qalaji, M, R., Naseem, M., Khan, S., Sohail, M.,2022. Recent developments and advanced strategies for promoting burn wound healing.
17. Ihsan ,M., Rahmadian ,R., Raymond ,B., 2021. Comparison of Burn Wound Histopathology Imaging between Epidermal Growth Factor Spray andSilver Sulfadiazine Application: An in Vivo Study.
18. Kemkes. Tata Laksana Luka Bakar. Diakses dari https://yankes.kemkes.go.id/unduh/fileunduhan_1610415947_8432_37.pdf
19. Kristyaningsih, P., 2016. Efektivitas Lidah Buaya (Aloe Vera) Dan Daun sirih (Piper Betle Linn) Terhadap Kesembuhan Luka Bakar Derajat II Pada Tikus Putih (Rattus Norvegicus Strain Wistar) Di Peternakan Tikus Sidomulyo Kediri. Jurnal Ilmu Kesehatan. 5: 114–121.
20. Maenthaisong ,R.,Chaiyakunapruk, N., Nirutraporn, S., Kongkaew, C., 2007. The Efficacy of Aloe Vera Used for Burn Wound Healing: A Systematic Review.
21. Minori ,N., 2019.Effect of Aloe Cream versus Silver Sulfadiazine for Healing Burn Wounds in Rats .
22. Muharraran ,F., Rusip ,G., Dalimunthe ,R ,P., 2022. Potential of Hydrogel Acemannan Aloe Vera on Wound Healing After Tooth Extraction In Vivo Via Regulation of Inflammatory Respons.
23. Pardina ,N ,A., Setyowatie ,L., 2020. Peran Astaxanthin Pada Luka Bakar.
24. Parihar ,A., Parihar , M, S., 2008. Oxidative stress and anti-oxidative mobilization in burn injury.
25. Putra, O ,N., 2022. Effects of Empirical Antibiotics Adminitration on the Level of CReactive Protein and Inflammatory Markers in Severe Burn Patients.

26. Putri ,I ,N ,W., 2019. Perbandingan Efektivitas Silver Sulfadiazine dan Madu dalam Penyembuhan Luka Bakar.
27. Rochmah, W., Holidah., DMaria, F., 2017. Pengaruh Pemberian Sari Buah Kurma (*Phoenix dactylifera*) terhadap Kadar Malondialdehid (MDA) Mencit Balb/c yang Dipapar Asap Rokok. Universitas Jember [Preprint]
28. Rusip ,G., Ilyas ,S., Lister ,N ,E., 2022. The Effect of Indigestion of Red Dragon Fruit Extract on Levels of Malondialdehyde and Superoxide Dismutase After Strenuous Exercise in Rats (*Rattus Norvegicus*).
29. Salah ,F., Ghoul,Y, E., Mahdhi, A., Majdoub, H., Jarroux, N., Sakli, F., 2017. Effect of the Deacetylation Degree On The Antibacterial and Antibiofilm Activity of Acemannan from Aloe Vera.
30. Shupp , J, W., Nasabzadeh, T, J., Rosenthal, D, S., Jordan, M, H., Fidler, P., Jeng ,J, C., 2010. A Review of The Local Pathophysiologic Bases of Burn Wound Progression.
31. Singer, A. J., Boyce, T. S., 2018. Burn Wound Healing and Tissue Engineering. HHS Public , 1-13.
32. Sipahutar, R., 2020. Gambaran C-Reactive Protein (CRP) pada perokok Aktif.
33. Somboonwong, J., Thanamitramanee, S., Jariyapongskul, A., Patumraj. S., 2000. Therapeutics Effects of Aloe Vera On Cutaneous Microcirculation and Wound Healing in Second Degree Burn Models in Rats.
34. WHO :WHO|Burns. 2018. accessed Oktober 2022
35. Wulandari, E., 2016 .Efek Ekstrak Kulit Buah Rambutan terhadap Kadar MDA dan SOD Tikus yang Dipapar Asap Rokok. Universitas Negeri Semarang [Preprint]
36. Yaman ,I., Durmus, A, S., Ceribasi, S., Yaman, M., 2010. Effects of Nigella Sativa and Silver Sulfadiazine on Burn Wound Healing in Cats.
37. Yulianti ,A ,B., Sumarsono, S, H., Ridwan, A., Yusuf, A, T., 2015. Hubungan Reactive Oxygen Species (ROS) dan Superoxide Dismutase (SOD) pada protein A- Sinuklein larut air pada batang otak tikus yang diinduksi Rotenon.
38. Zhang ,J., Fu, Y., Yang, P., Liu, X., Li, Y., Gu, Z., 2020. ROS Scavenging Biopolymers for Anti-Inflammatory Diseases: Classification and Formulation.
39. Zhou ,H., Fang, Q., Li ,N., Yu, M., Chen, H., Guo ,S., 2021. ASMq protects against early burn wound progression in rats by alleviating oxidative stress and secondary mitochondria - associated apoptosis via Erk.p90RSK/Bad pathway.