

The effect of Quran recitation on t-cell lymphocyte activity in mice model of breast cancer



Akbar Reza Muhammad¹, Yunyastiti D. Palupi¹, Mega Astri¹, Hafid Algristian^{1*}

ABSTRACT

Introduction: Breast cancer is the leading cause of death in women. Many studies of adjuvant therapy have used the Quran recitation (later will be mentioned as the murotal approach). The murotal approach has been shown to induce feelings of well-being, enhance therapeutic response, and aid in patient healing. This study aimed to analyze the effect of the murotal approach on lymphocytic cell activity in inducing programmed cancer cell necrosis in a breast cancer mouse model.

Methods: After testing in a fully randomized design using 24 female mice (*Mus musculus*) BALB/c strain, a real-world experimental study with a control group design method. The mice were divided into four groups: negative control (K), positive control (K+), treatment group (P1) which receiving the murotal approach for 2 hours and 30 minutes per day for a week, and the (P2) which receiving the murotal approach for 30 minutes in five times a day for a week. Mice were subcutaneously injected with DMBA 0.56 mg/20 g every 2 days for 3 weeks. The murotal approach was using specifically in *Surah al-Faatihah and Al-Baaqarah* from *Qori' Al-Mathrud*. Cancer tissue was harvested 5 weeks after her. The data were analyzed using the Kruskal-Wallis hypothesis test.

Results: There was a significant difference ($P < 0.05$) in the effect between the P2 treatment group that received murotal therapy for 30 minutes at each prayer time and the other groups. The efficacy of treatment murotal for 2 hours and 30 minutes per day does not affect the extent of lymphocytic infiltration.

Conclusion: From the research, the dosage of murotal therapy to increase the degree of lymphocyte infiltration is for 30 minutes five times a day, or during prayer time.

Keywords: breast cancer, Quran, murotal, lymphocyte.

Cite This Article: Muhammad, A.R., Palupi, Y.D., Astri, M., Algristian, H. 2022. The effect of Quran recitation on t-cell lymphocyte activity in mice model of breast cancer. *Bali Medical Journal* 11(3): 1111-1115. DOI: 10.15562/bmj.v11i3.3473

¹Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia;

*Corresponding author:
Hafid Algristian;
Universitas Nahdlatul Ulama Surabaya,
Surabaya, Indonesia;
dr.hafid@unusa.ac.id

Received: 2022-07-02
Accepted: 2022-08-10
Published: 2022-09-09

INTRODUCTION

Cancer is one of the leading causes of death throughout the world. Based on GLOBOCAN data, the International Agency for Research on Cancer (IARC), it is known that in 2020 there were 2,261,419 new cases of cancer and 684,996 deaths from cancer worldwide. Breast cancer ranks first as the most common type of cancer suffered by women in the world. Breast cancer contributed 11,7% of the total new cases of overall cancer diagnosed in 2020.¹

Data from *Riset Kesehatan Dasar* (basic health research of Indonesia), stated that the national prevalence rate of cancer was 1.4 per 1000 population in 2013 and increased to 1.8 per 1000 population in 2018. If the figure is projected on the population of East Java with a population of around 39 million, it is estimated that

there is an increase in cancer patients from around 55,000 to 72,000 cancer patients.² Based on GLOBOCAN data, the International Agency for Research on Cancer (IARC), it is known that in 2020 there were 65,858 or 16,6% of all cancer new cases and 22,430 or 9,6% of all deaths from cancer in Indonesia. The estimated incidence of breast cancer in Indonesia is 148,11 per 100,000 women.³

Carcinogenesis is a multistage and multistep process involving modification and mutation to genes that regulate normal cellular function including cell growth control processes.⁴ Compounds that can cause cancer include compounds of Polycyclic Aromatic Hydrocarbon (PAH), such as 7,12-dimethylbenz(a) Anthracene (DMBA) whose metabolites can bind to DNA. Overall, the carcinogenesis process can be divided into three multi-steps process of cancer, which

are initiation, promotion, and progression. Furthermore, carcinogenesis causes the conversion of malignant originating from benign hyperplastic cells into malignant cells that can invade and metastasize, a manifestation of advanced genetic and epigenetic changes.⁵

Various therapeutic approaches have been carried out. Surgery, radiotherapy, chemotherapy, and targeted therapy are common cancer treatments, but many patients experience recurrence after therapy. Some studies have shown an increase in mortality and the risk of metastases when cancer recurrence occurs after surgery therapy.⁶ While chemotherapy used to prevent cancer cell progression is not without side effects, a cohort study showed at least one side effect of 86% during the study period with increasing risk according to age.⁷ Talk therapy, otherwise known

as psychotherapy in mental health, has been shown to improve patients' response to chemotherapy and reduce the risk of side effects. Until now, there is no known biological mechanism why non-biological therapy can have a biological impact, especially in cancer cases.⁸

Centuries ago, traditional eastern medicine had proposed the hypothesis that the mind and body were thought to be interrelated. Nowadays, Theorists propose that stressful events trigger cognitive and affective responses that trigger, induce sympathetic nervous system and endocrine changes, and ultimately impair immune function. The relationship with cancer shows that: (1) Emotional stress affect the function of the immune system, and, ultimately, cancer processes.⁹ (2) Psychological Stress can increase the body's susceptibility to carcinomas.¹⁰ Recently, a new opinion has been put forward that there are differences in the effects of acute and chronic stress on the overall immune system. Acute stress has been shown to increase dendritic cells, neutrophils, macrophages, and lymphocyte recruitment, maturation, and function, which has been shown to enhance innate and adaptive immune responses by activating and modulating memory T cells. Unfortunately, chronic stress can dysregulate innate and adaptive immune responses by altering the balance of type 1–type 2 cytokines and suppressing immunity by decreasing the number, function, and recruitment of leukocytes.¹¹ Silberman and colleagues show that stress exerts a differential effect on T-cell-dependent antibody production. IgG production is augmented after acute stress and impaired in a chronic situation.¹² Endogenous glucocorticoids also known to cause dysfunction of CD8+ T cells, characterized by increased expression of PD-1, which functions as a negative regulator of the immune response.¹³ Psychological stress, also known to induce activation of 2-adrenergic receptors (B2AR), cause increased expression of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and Tregs. These conditions have triggered the transition of the anti-tumor immune response to a pro-tumor immune response.¹⁴

With the need for a better cancer

therapy approach, supported by evidence of the effectiveness of the mural approach on immune system regulation, as well as evidence of the success of the cancer immunotherapy approach by two Nobel Prize winners in 2018, namely James P. Allison and Tasuku Honjo through the theory of Immune checkpoint inhibitors, two proteins that different, namely CTLA-4 and PD-1,¹⁵ researchers want to prove the concept of applying Cancer Immunotherapy with the mural approach to improve the effectiveness of lymphocyte cells in improving cancer grade in breast cancer mice models. This study aimed to analyze the effects of the mural approach on lymphocyte cell activity in inducing programmed cancer cell necrosis in breast cancer mice models.

METHODS

General Background of Research

This study was an experimental study. The type of study used in this study was a true experiment using laboratory animals in the form of female mice aged 10-12 weeks (*Mus musculus* strain BALB/c). The study design used was a purely post-test control group design. The inclusion criteria for mice were 10-12 weeks of age, and the exclusion criteria for mice were birth defects and pain.

Sample of Research

The number of samples in this study was calculated based on the number of groups in the parent study. Since there are 4 groups, then based on the Federer formula (1977) the minimum number of the sample is 6 samples. Due to the number of groups being 4, then the total number of mice is 24.

Instrument and Procedures

Mice were given in 4 groups: K- (untreated control), K + (with DMBA injection without mural approach), P1 (DMBA injection for 3 weeks, then mural approach in 2.5 hours 1) Weekly) and P2 (1 week). After 3 weeks of DMBA injection, the mural approach was played at each prayer time (5 times a day) for 30 minutes for 1 week. According to Federer's formula, the number of mouse samples required is 24. This is because the number of groups is 4, so the number of mouse samples used in

this study is 6 per group. Each group was then placed in a plastic cage with a sound source on top of the cage at an effective distance of approximately 40 cm. Mice were subcutaneously injected with DMBA 10 mg/kg body weight at 2-day intervals for 3 weeks. Mice can be heard reading *Qori' Al-Mathrud's Alquran Suratal-Faatihah and Al-Baaqarah*. The mice were then observed for 10 days to see if there were lumps in the chest of the mice, the duration of the test was calculated, and the cancerous tissue was harvested after 5 weeks.

Data Analysis

Data were analyzed with the *Kruskal-Wallis* and *Mann-Whitney* hypothesis test. Analysis was carried out using SPSS ver. 24.

RESULTS

Degree of Lymphocyte Infiltration

The breast slides were analyzed in the laboratory of Universitas Hang Tuah Surabaya and Universitas Nahdlatul Ulama Surabaya. We observed lymphocyte infiltration and signs of malignancy in the histology of the mice breasts. From the observations, it was found that the average percentage of breast histopathology was as follows.

Figures 1 and 2 has showing that maximum results were obtained for the mural approach for 30 minutes each prayer time where the percentage of P2 group lymphocyte infiltration appeared more than the other groups. From Figure 5.1 it is also known that the picture of P2 lymphocyte infiltration has a picture of lymphocyte infiltration in severe degrees. Then from these data, the *Kruskal-Wallis* hypothesis test with SPSS was carried out and the following results were obtained.

It can be seen in the Table that the value of probability (Sig.) <0.05 so that there is a minimum result of a pair of differences between the 5 groups tested both from K-, K +, P1, and P2. Furthermore, to find out which groups are different, a nonparametric test will be conducted using the Mann-Whitney test.

Based on the table above, the results are as follows:

1. Comparison between K-groups with K + there is no significant difference,

- both groups can be used as a control group.
- Comparison between K + and P1 groups did not have a significant effect where the degree of lymphocyte infiltration did not increase even though the DMBA injection was given.
 - Comparison between K + and P2 groups has a significant effect where the degree of lymphocyte infiltration has increased which is seen in the P2 group after being given the murotal approach for 30 minutes each prayer time.
 - In comparison between groups P1 and P2 there is a significant difference in effect where the degree of lymphocyte infiltration has increased in the P2

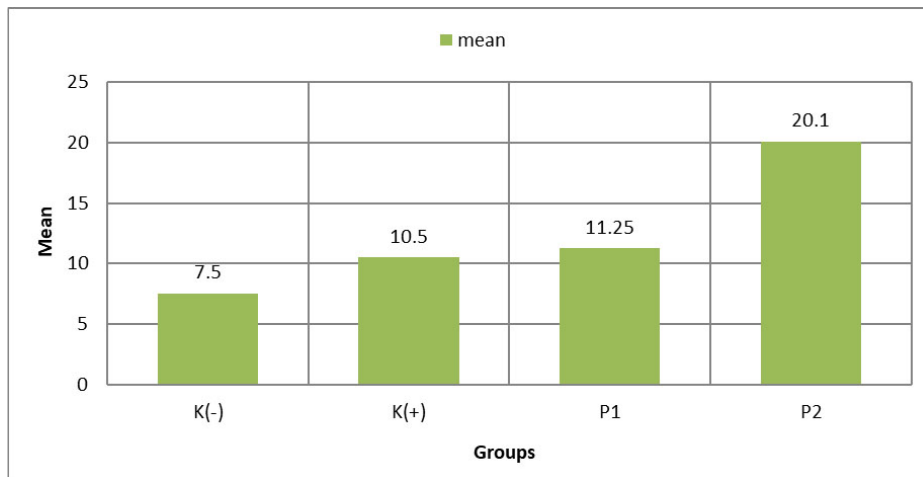


Figure 1. Diagram of the average lymphocyte infiltration percentage of breast histopathology.

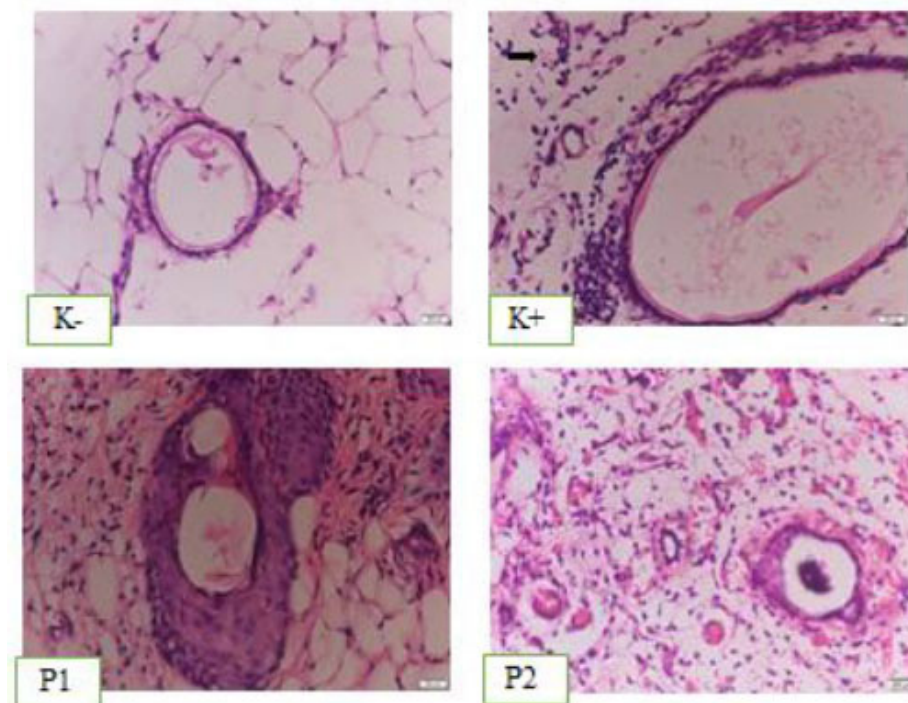


Figure 2. Mice Breast Histopathology slide with 40x magnification. Description: Negative Control Mice (K-), Positive Control Mice/given injection of DMBA 10mg/kg BW (K+), Treatment mice were given DMBA injection for 3 weeks then the murotal approach in 2 and a half hours for 1 week (P1), Mice were given injection DMBA for 3 weeks then read the murotal approach in 30 minutes every prayer time for 1 week (P2), HE coloring.

group after being given the murotal approach for 30 minutes each prayer time rather than giving for 2 hours 30 minutes every day.

From the results of the table above it can be concluded that the effect of the murotal approach is given for 30 minutes each prayer time in a day against the degree of lymphocyte infiltration. This therapy is considered to have an effective dose to increase lymphocyte infiltration in its ability to prevent worsening of the stage of breast cancer.

DISCUSSION

Observations and Research Subjects

Observation results showed that mice induced by the murotal approach tended to be calmer and not more aggressive than other groups. While the negative control group tends to be more aggressive and hyperactive as evidenced by the behavior and activity of mice during the research observation.

This can be caused by the murotal approach which can activate the amygdala's emotional center in controlling positive emotions. The amygdala is an area of conscious behavior that works at the subconscious level. The signal received by the amygdala will then be transmitted to the hypothalamus and will affect the performance of the autonomic nervous system. Autonomic nerves will affect the contraction and relaxation of organs.¹⁶

At the time of observation also found fluctuations in body weight of mice due to the influence of the body response of mice. These fluctuations may be caused by DMBA injection treatment which can cause organic changes in cellular and systemic levels and the provision of the murotal approach which can increase the appetite of mice. This is to previous studies which stated that Cachexia syndrome is characterized as a decreased body weight profile due to cancer progression. Cachexia in mice is thought to be caused by abnormal metabolites produced during the development of tumor periods both by the immune system and by cancer itself.¹⁷

According to the national academy of science, stating that clinical signs of behavior changes to abnormal due to injury and stress can cause changes in feed and drinking water consumption

Table 1. Kruskal Wallis test for the degree of lymphocyte infiltration.

Group	Total (n)	Kruskal Wallis Test	Mean rank
K-	4	P < 0,05	7,50
K+	4		10,50
P1	4		11,25
P2	4		20,10

Table 2. Mann-Whitney Test Degree of Lymphocyte Infiltration.

Group	P2	P1	K+	K-	Lymphocyte Infiltration
P2					20,10
P1	0,030				11,25
K+	0,009	0,937			10,50
K-	0,004	0,140	0,394	-	7,50

Asymp. Sig. < 0,05 = significant

habits, accumulation of reddish-brown exudates around the eyes and nostrils, weight loss, decreased activity, bending posture, piloerection, poor grooming habits, difficult breathing and vocalization, increased or decreased aggressiveness and self-mutilation.¹⁸

Degree of lymphocyte Infiltration

The results showed the effect of giving the murotal approach to an increase in the degree of lymphocyte infiltration. In this study, the highest percentage value of lymphocyte infiltration was in the P2 group where mice were given the murotal approach for 30 minutes in five prayer times, while the lowest percentage was obtained by group K (-) who were not given DMBA injection or the murotal approach. From the results of the hypothesis test, there were significant differences in influence between the four groups.

From the results of the research shown in [Figure 2](#), we can find differences in the picture of lymphocyte infiltration based on histopathology using Hematoxylin Eosin (HE) staining. On examination using 40x magnification can be found as severe lymphocyte infiltration in the P2 group and mild grade in group K (-). Giving the murotal approach for 30 minutes at each prayer time is considered to give the best results compared to the other groups. The increase in lymphocyte infiltration in this group showed an increase in an adequate immune response to fight cancer-causing foreign substances and mutation of breast gland cells, thereby increasing the

prognosis and repair of tissue in the breast gland.

Previous results suggest that the incidence of Tumor Infiltrating Lymphocytes (TIL) in breast cancer patients chemotherapy has an important role in mediating the response of chemotherapy and increasing clinical outcomes in all breast cancer subtypes. The immune adaptive response to breast cancer can be seen in breast infiltration lesions starting from the beginning as benign tumors that increase in density as the tumor develops into malignancy.¹⁹

Another study shows that there is a link between high lymphocyte infiltration and young women. The results of research on all breast cancer subtypes show that the composition and magnitude of lymphocyte infiltration affect clinical outcomes better and show that breast cancer is an immunogenic tumor.²⁰

The different effects of the murotal approach

The results of the study of differences in the effect of the murotal approach can be seen in bivariate analysis using the Kruskal Wallis test in [table 1](#). The results of the study found a difference in the effect of the murotal approach on the increase of lymphocyte infiltration rates in each treatment group (P < 0.05). The results of the Mann-Whitney follow-up test shown in [Table 2](#) found differences in the effect of the degree of infiltration of P2 lymphocytes on all other treatment and control groups. The existence of

differences in influence in this study can be caused due to the provision of the murotal approach for 30 minutes each prayer time can affect the quality of the body's defense system at the hormonal and molecular levels. Giving therapy to the P2 group was indicated according to one of the principles of immunotherapy in cancer treatment by increasing the performance of the immune system and strengthening it against the forerunner of cancer cells that are progressively growing and developing.²¹

The effect of giving the murotal approach and immune system enhancement has been discussed before in the Sholeh study (2008) in the viewpoint of psychoneuroimmunology which states that the application of worship can bring positive feelings that activate the amygdala. The amygdala sends information to the locus ceruleus so that it activates an autonomic nerve reaction through the hypothalamus. Furthermore, the hypothalamus secretes neurotransmitters, endorphins, and enkephalins which function as painkillers and control excessive CRF secretion. As a result, HPA-axis will be controlled to stabilize ACTH secretion. Decreasing ACTH will stimulate a decrease in the production of the hormone cortisol in the adrenal cortex. Decreased cortisol production can increase other immune systems such as T-CD4, CD8 +, and CD56 + NK lymphocytes to prevent the spread of certain diseases such as breast cancer and HIV AIDS. If the administration of the murotal approach is carried out continuously using 30 minutes of therapy every time of prayer, cortisol levels will be more regular to reduce the inhibition of lymphocyte cell production.²¹

The murotal approach was able to generate brain waves, namely alpha waves that have a frequency of 7-14 Hz and delta waves that have a frequency of 0.5-4 Hz. Alpha waves are identical to relaxation. Alpha waves are also called relaxed awareness. Delta waves are often found in the phase of deep sleep. These waves are also called waves of the resting phase of the body and mind. This resting brain phase is able to reverse depression, increase serotonin and BDNF,²² thereby increasing self-healing potential. These

waves can make our bodies carry out self-healing processes, repair tissue damage and produce new cells. The two waves synergize with each other to help immune cells, especially lymphocytes, to effectively attack cancer cells.²³ The spirituality factor that increased when the murotal approach also gives positive reactions that can improve mood and happiness, so it also affects improving the immune system.¹⁹

CONCLUSION

Based on the results of this study, we conclude that administration of the murotal approach for 30 minutes at each prayer time affects the level of lymphocytic infiltration with the administration of the murotal approach for 2 hours and 30 minutes per day can do. time. The effect of the murotal approach for 30 minutes at each prayer time affects the extent of lymphocyte infiltration compared to other groups. There was a significant difference in the effect between the P2 treatment groups that received the murotal approach for 30 minutes at each prayer time with the other groups ($P < 0.05$). The effect of treatment with the murotal approach for 2 hours 30 minutes per day does not affect the degree of lymphocytic infiltration. According to research, the dosage of the murotal approach to increase the degree of lymphocyte infiltration is 30 minutes of the murotal approach five times a day, or each prayer time.

FUNDING

The authors are responsible for all of the study funding without a grant or any external funding source.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTION

All authors similarly contribute to the think about from the investigation concepts, information acquisitions, information investigation, factual investigations, changing the paper, until detailing the consider comes about through publication.

ETHICAL CONSIDERATION

The investigators agreed to conduct this study in full agreement with the principles of the Declaration of Helsinki and its subsequent related amendments. This study was approved by the Ethics Committee of the Surabaya Islamic Hospital. This research also has put forward ethics first and has passed the ethical test following international ethical standards for the animal experiment (No. 02/MEC/KEPUHT/2019)

ACKNOWLEDGMENTS

Authors expressed their acknowledgment to Direktorat Jendral Pendidikan Tinggi (Dirjen DIKTI) Republic of Indonesia as the main sponsor for the research has been granted in the student creativity program competition.

REFERENCES

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.* 2021 May;71(3):209-249.
- Kementerian Kesehatan RI. Riset kesehatan dasar (RISKESDAS). Jakarta. 2018.
- The Global Cancer Observatory. Cancer Incident in Indonesia. 2020.
- Klaunig JE. Carcinogenesis, *An Introd. to Interdiscip. Toxicol.* pp. 97–110. 2020.
- Basu AK. DNA Damage, Mutagenesis and Cancer. *Int J Mol Sci.* 2018 Mar 23;19(4):970.
- Dent R, Valentini A, Hanna W, Rawlinson E, Rakovitch E, Sun P, Narod SA. Factors associated with breast cancer mortality after local recurrence. *Curr. Oncol.* 2014; 21(3):p. e418.
- Pearce A, Haas M, Viney R, Pearson S, Haywood P, Brown C, Ward R. Incidence and severity of self-reported chemotherapy side effects in routine care: A prospective cohort study. *PLoS One.* 2017; 12(10):p. e0184360.
- Algristian H, Wibowo R, Ishardyanto H, and Maramis MM. Do the Effectiveness of Psychotherapy to Women Patients with Locally Advanced Breast Cancer (LABC) is affected by Personality Traits and Meaning of Illness? in *Konferensi Nasional II Psikiatri Religi dan Spiritual.* 2017.
- Thornton LM, and Andersen BL. Psychoneuroimmunology examined: The role of subjective stress. *Cellscience.* 2006; 2(4): p. 66.
- Kruk J, Aboul-Enein BH, Bernstein J, and Gronostaj M. Psychological Stress and Cellular Aging in Cancer: A Meta-Analysis. *Oxid. Med. Cell. Longev.* 2019; vol. 2019.
- Dhabhar FS. Enhancing versus Suppressive Effects of Stress on Immune Function:

- Implications for Immunoprotection versus Immunopathology. *Allergy, Asthma Clin. Immunol.* 2008; 4(1):pp. 1–10.
- Silberman DM, Wald MR, and Genaro AM. Acute and chronic stress exert opposing effects on antibody responses associated with changes in stress hormone regulation of T-lymphocyte reactivity. *J. Neuroimmunol.* 2003; 144(1): pp. 53–60.
 - Tian W, Liu Y, Cao C, Zeng Y, Pan Y, Liu X, Peng Y, Wu F. Chronic Stress: Impacts on Tumor Microenvironment and Implications for Anti-Cancer Treatments. *Front Cell Dev Biol.* 2021 Nov 19;9:777018.
 - Zhang L, Pan J, Chen W, Jiang J, Huang J. Chronic stress-induced immune dysregulation in cancer: implications for initiation, progression, metastasis, and treatment. *Am J Cancer Res.* 2020 May 1;10(5):1294-1307.
 - Fares J, Fares MY, and Fares Y. Immune checkpoint inhibitors: Advances and impact in neuro-oncology. *Surg. Neurol. Int.* 2019; 10(1).
 - Dewa Ayu Gede Putri Saraswati. Pengaruh terapi musik relaksasi instrumental terhadap tingkat kecemasan pasien stroke di ruang HCU BRSU Tabanan. *Univ. Udayana.* 2013.
 - Rosani Senawati. Pengaruh Produk Daun Cincau Hijau Cyclea Barbata L. Miers Dan Premna Oblongifolia Merr Terhadap Kapasitas Antioksidan Sel Limfosit Mencit C3H Bertumor Kelenjar Susu. *Inst. Pertan. Bogor.* 2003.
 - National Academic of Science (NAS). *Laboratory Animal Management Rodents. Committee on Rodents, Institute of Laboratory Animal Resources, Commission on Life Sciences National Research Council.* Washington DC: National Academy Press.
 - Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, Wienert S, Van den Eynden G, Baehner FL, Penault-Llorca F, Perez EA, Thompson EA, Symmans WF, Richardson AL, Brock J, Criscitiello C, Bailey H, Ignatiadis M, Floris G, Sparano J, Kos Z, Nielsen T, Rimm DL, Allison KH, Reis-Filho JS, Loibl S, Sotiriou C, Viale G, Badve S, Adams S, Willard-Gallo K, Loi S; International TILs Working Group 2014. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol.* 2015 Feb;26(2):259-71.
 - Dendreon. Cancer Immunotherapy: fundamental concepts and emerging role. *Oncol. Perspect.* 2013.
 - Sholeh M. *Terapi shalat tahajud.* Jakarta: mizan publika. 2008.
 - Azizah NAS, Veterini L, Algristian H, and Salim HM. Expression of Brain-Derived Neurotrophic Factor in the Brain of Depressed Mice: Systematic Literature Review. *Qanun Med. - Med. J. Fac. Med. Muhammadiyah Surabaya.* 2022; 5(2): pp. 189–203.
 - Mustajib A. *Rahasia Dahsyat Terapi Otak.* Jakarta: PT. Wahyu Media, 2010.



This work is licensed under a Creative Commons Attribution