**Effectiveness of Nigella sativa Addition against TNF-Alpha in Stage III and IV Breast Cancer Undergoing Doxorubicin and Cyclophosphamide Chemotherapy at Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia**

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**ABSTRACT**

**Background:** Breast cancer is a malignancy caused by the continuous uncontrolled growth of the component cells of the ducts or lobules of the breast gland. Globally, breast cancer is the second leading cause of death in women. *Nigella sativa* contains substances thymoquine. Thymoquinone can inhibit the progression of cancer through anti-inflammatory, anti-oxidant mechanisms, inhibit proliferation, trigger apoptosis, and prevent angiogenesis. Chronic inflammation in breast cancer is mediated by one of them is TNF-alpha tumors. Elevated levels of TNF-alpha have a significant association with poor prognosis and progression of breast cancer. This study aims to determine the effectiveness of the additional *Nigella sativa* in declining TNF-alpha levels in breast cancer patients at Dr. Mohammad Hoesin General Hospital Palembang.

**Methods:** This research used a randomized, open clinical trial design, which was carried out at the internal medicine hematology-oncology medical polyclinic and the surgical oncology polyclinic of Dr. Mohammad Hoesin General Hospital Palembang from January 2023 to October 2023. Data processing for data analysis used SPSS version 26 for Windows.

**Results:** There were 36 research subjects followed during the study period and received Doxorubicin chemotherapy and cyclophosphamide (AC), which were divided into treatment groups of 18 people (chemotherapy with the addition of *Nigella sativa* @600 mg 2x2 capsule/day and a control group of 18 people. A TNF-alpha examination was carried out before and after to assess the comparison before and after. From the results, it was found that TNF-alpha levels in the treatment group showed a decrease in TNF-alpha levels in breast cancer patients receiving AC chemotherapy at Dr. Mohammad Hoesin General Hospital, Palembang.

**Conclusion:** The addition of *Nigella sativa* has the effect of reducing TNF-alpha in breast cancer patients receiving AC chemotherapy at Dr. Mohammad Hoesin General Hospital, Palembang.

1. **Introduction**

Globally, breast cancer is the second leading cause of death in women. The incidence of breast cancer reaches 2.3 million, and the mortality rate is 683 thousand per year. The number of new cases of breast cancer reached 68,858 cases, which covers 16.6% of cancer cases in Indonesia, with a mortality rate of 22 thousand cases. This chronic inflammation is
mediated by various cytokines, chemokines, and growth factors, one of which is tumor necrosis factor-α (TNF-α). In large and chronic amounts, TNF-α can cause chronic inflammation, thereby increasing the risk of death in breast cancer patients and increasing disease progression due to the anti-apoptotic and mitogenic effects of TNF-α, resulting in increased cancer cell proliferation and metastasis. In addition, TNF-α is also reported to have an important role in the tumor microenvironment (TME), which has a major influence on the development and evolution of the disease. In the TME, TNF-α is secreted by stromal cells, especially by M1 TAMs, and by cancer cells Macrophages and the breast cancer tumor microenvironment.\textsuperscript{1-4}

AC chemotherapy is one of the treatment options for breast cancer patients. AC chemotherapy includes administration of doxorubicin and cyclophosphamide. Various studies have reported the influence of TNF-α on the sensitivity of cancer cells to AC chemotherapy. This is based on the role of TNF-α in chemoresistance. TNF-α levels are positively correlated with breast cancer cell resistance to doxorubicin, which is a component of AC chemotherapy. TNF-α levels are positively correlated with breast cancer cell resistance to doxorubicin, which is a component of AC chemotherapy. Nigella sativa, also known as black cumin (black cumin), is a dicotyledon from the Ranunculaceae family and has been used for two millennia as an appetizer, flavoring agent, and nutritional and nutraceutical agent in various societies in Asia, Africa, and Europe. Interestingly, Nigella sativa is an example of herbal medicine that is quite well-known and widely used. Extract from \textit{N. sativa} seeds contains various substances such as thymoquinone (TQ), proteins, alkaloids such as nigellicine and nigelidine, saponin in the form of alpha-hederin, fatty acids, fatty oils, and essential oils where thymoquinone is the active substance of \textit{Nigella sativa}. Thymoquinone can inhibit the progression of cancer through anti-inflammatory, anti-oxidant mechanisms, inhibit proliferation, trigger apoptosis, and prevent angiogenesis.\textsuperscript{5-8} This study aims to determine the effectiveness of the addition of \textit{Nigella sativa} in patients receiving AC chemotherapy against declining TNF-α levels in breast cancer patients at Dr. Mohammad Hoesin General Hospital, Palembang.

2. Methods

This research is an experimental research design, a randomized, open clinical trial. A total of 36 subject studies participated in this study, where the research subjects met the inclusion criteria. The inclusion criteria for this study were age ≥18 years, diagnosed through histopathological examination and treatment at the hematology-oncology medical polyclinic and the inpatient ward of the internal medicine department of Dr. Mohammad Hoesin General Hospital Palembang which was divided into intervention and non-intervention groups (premenopausal and postmenopausal, metastatic patients and nonmetastatic), breast cancer patients who will undergo group chemotherapy Anthracycline, and Alkylating agent and are willing to take part in the research by signing the form informed consent. Research subjects were grouped into two groups, namely, the treatment group and the placebo group. Treatment groups are assigned \textit{Nigella sativa} 2x1200 mg capsules and receive 4 series or 12 weeks of AC chemotherapy. The placebo group was given 2x2 placebo capsules and received 4 series or 12 weeks of AC chemotherapy. This study has received approval from the medical and health research ethics committee of Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia.

This study made observations on sociodemographic, clinical, and laboratory data. TNF-α examination was carried out using the ELISA method (Human TNF-α HS Elisa, E-EL-H0109) using blood samples in serum preparations according to the ELISA kit instructions. The data that has been collected will be processed using the statistical package for the social sciences (SPSS) version 26.0 program, which is displayed in the form of narratives, tables, and graphs. The statistical test will be carried out by descriptive analysis and a normality test using
the Shapiro-Wilk test. Numerical variables that have a normal distribution will be presented with the mean and standard deviation, while those that do not have a normal distribution will be presented in the form of a median and range. Categorical data is presented in percentage form. The data that will be presented in the frequency distribution table of the basic characteristics of research subjects are variable data: Age, education level, body mass index, breast cancer stage, and serum TNF-α levels. To calculate the meaningful value of the relationship between gifts *Nigella sativa* with TNF-α levels, a hypothesis test will be carried out using the analysis of covariate (ANCOVA) test with the baseline TNF-α covariate and the independent variable *Nigella sativa* and the outcome variable is TNF-α after administration of thymoquinone.

3. Results
The average age of research subjects in the treatment group was younger than the control group (48.88 and 50.55), and the average BMI in the treatment group was lower than the control group (21.48 and 21.91). Education level, cancer stage, and menopausal status in both groups were similar. The initial mean TNF-α value in the treatment group was higher than in the control group (0.69 and 0.57), but at the end of the study, the mean TNF-α value was higher in the control group (0.49 and 0.49). The average Hb value both at the start of the study and at the end of the study was higher in the treatment group compared to the control group (11.51 and 10.69 and 11.23 and 10.66). The average leukocyte values both at the start of the study and at the end of the study were higher in the treatment group compared to the control group (7,444 and 9,360 and 6,571 and 10,047). The initial average erythrocyte value in the treatment group was lower than in the control group (3.79 and 3.96), but at the end of the study, the average erythrocyte value was lower in the control group (3.95 and 3.94). The initial mean platelet value in the treatment group was higher than in the control group (380,000 and 368,944), but at the end of the study, the mean platelet value was higher in the control group (357,277 and 380,777). The initial average Ca-153 value in the treatment group was higher than in the control group (208.44 and 183), but at the end of the study, the average Ca-153 value was higher in the control group (63.84 and 97.22). The average QOL scores both at the start of the study and at the end of the study were lower in the control group (63.55 and 61.94) compared to the treatment group (68 and 65.5).

In this study, the average TNF-α level before treatment in the initial treatment group was 0.69 (0.36-1.55), and in the control group was 0.57 (0.18-1.93). After treatment with the administration of *Nigella sativa*, the results showed that in the treatment group, the average level of TNF-α decreased to 0.43 (0.24-1.08) with statistically significant results (p<0.05). Different results were shown in the control group, where there was a decrease in the average level of TNF-α to 0.49 (0.23-0.78), but this was not statistically significant (p>0.05). The Mann-Whitney test was carried out to assess the difference in mean levels of ΔTNF-α (End TNF-α- Early TNF-α) between the groups that received *Nigella sativa* with the control group, and the results were statistically significant (p < 0.05).

In this study, the average QOL level before treatment in the initial treatment group was 63.55 (58-70), and in the control group, it was 61.94 (56-68). After treatment with the administration of *Nigella sativa*, the results showed that in the treatment group, there was an increase in the average QOL level to 68 (61-76) with statistically significant results (p<0.05). The same results were shown in the control group, where there was an increase in the average QOL level to 65.5 (58-74), which was statistically significant (p<0.05). The Mann-Whitney test was carried out to assess the difference in the average level of ΔQOL (End QOL-Early QOL) between the groups that received *Nigella sativa* with the control group, and the results were not statistically significant (p > 0.05).
4. Discussion

*Nigella sativa* contains thymoquinone and other metabolites that have potential anticancer effects, especially in colorectal cancer. Studies have indicated that *Nigella sativa* has the potential to influence various genes and signaling pathways involved in colorectal cancer. Anticancer effects of *Nigella sativa* on breast cancer through the regulation of various miRNAs. This study analyzes the anti-cancer potential of extracts from *Nigella sativa* in vitro against MDA-MB-231, MCF-7 breast cancer cells and in vivo on tumor growth in rats after successful oral administration of *Nigella sativa* oil. This study highlights the use of powerful integrants [thymoquinone (TQ), carvacrol, and trans-anethole (TA)], which exhibit anti-cancer properties. The results
demonstrated a reduction in solid tumors in vivo and restoration of near-normal tissue section architecture from the tumor set treated with *Nigella sativa*, indicating improved anti-tumorigenic potential.\(^9\)-\(^{12}\)

Administration of thymoquinone in rheumatoid arthritis synovial fibroblast (RA-FLS) can suppress the production of cytokines IL-6 and IL-8, and the expression of ICAM-1, VCAM-1, and Cadherin-11, all of which are induced by TNF-α. On evaluation of the signaling path, it was discovered thymoquinone plays a role in inhibiting the p38 phosphorylation pathway and JNK activation induced by TNF-α, but has no inhibitory effect on the NF-κB pathway in RA-FLS. The process of inhibiting the p38 phosphorylation pathway and JNK activation is caused by the inhibition of apoptosis-regulated signaling kinase 1 (ASK1), where the inhibition is dose-dependent. Thymoquinone can inhibit tumor cell proliferation by triggering cancer cell apoptosis and reducing the activity of TNF-α and NF-κB. Thymoquinone is also reported to have antioxidant effects in experimental mice by suppressing the expression of inflammatory factors such as IL-1β, TNF-α, IFN-γ, and IL-6. This study identified phytochemical constituents in *Nigella sativa* leaves, including tannins, saponins, steroids, and cardiac glycosides. This study demonstrates a stable neuropilin complex with key signaling pathways relevant to anti-cancer therapy. This research also performed molecular docking, pharmacophore modeling, and virtual screening to reveal interactions with oncogenes, as well as molecular dynamics simulations to assess complex dynamics and stability.\(^{13}\)-\(^{16}\)

Salivary cytokine profile of breast cancer patients and its relationship with tumor clinicopathological characteristics. This study found that the cytokine content in saliva, including TNF-α, correlated well with the clinicopathological characteristics of breast cancer. Salivary TNF-α levels, along with other cytokines, were observed to be increased in advanced breast cancer and at the level of tumor differentiation. This suggests a potential link between TNF-α levels and breast cancer development, which could have implications for patients’ quality of life.\(^{17}\)-\(^{20}\)

5. Conclusion

*Nigella sativa* is effective as an additional chemotherapy therapy to reduce TNF-α levels in breast cancer patients receiving AC chemotherapy therapy at Dr. Mohammad Hoesin General Hospital Palembang.

6. References


