The effectiveness of multiflora honey to prevent hepatotoxicity in invasive ductal breast cancer patients with FAC chemotherapy

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ABSTRACT

Background: Breast cancer is an epithelial malignancy of the breast duct, which chemotherapy is one of the treatment modalities. Hepatotoxicity is known as one of the side effects of chemotherapy. Multiflora honey provides hepatoprotective effects through its phenolic acid and flavonoid compounds. This study will evaluate the effect of Honey in preventing hepatotoxicity in ductal invasive breast cancer patients receiving FAC chemotherapy.

Methods: An experimental study with double-blind, randomized pre and post-test with control group design. About 36 ductal invasive breast cancer patients were divided into 2 groups, the control group who received FAC chemotherapy and the treatment group who received chemotherapy and 90 ml/day honey consumption for 14 days. The patient’s Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), and Gamma-Glutamyl Transferase (GGT) enzyme levels before and after 14 days of the study were assessed. Data were analyzed using SPSS version 15 for Windows by Mann-Whitney test and Wilcoxon tests.

Results: There was a significant difference in AST (33.72±44.35 U/L vs. 23.22±4.48 U/L; p<0.001) and ALT (29.72±16.96 U/L vs. 26.06±9.19 U/L; p=0.020) levels in the treatment vs. control group. In addition, a significant difference of ALP (107.00±65.89 U/L vs. 103.17±35.30 U/L; p=0.035) and GGT (56.61±58.16 U/L vs. 51.33±27.50 U/L; p=0.035) levels in the treatment vs. control group was also found in this study.

Conclusion: Multiflora honey has a hepatoprotective effect for invasive ductal breast cancer patients receiving FAC chemotherapy.

Keywords: Multiflora honey, Hepatotoxicity Induced Chemotherapy, Breast Cancer.


INTRODUCTION

Breast cancer is a malignancy of the epithelium of the breast ducts and lobules.¹ This malignancy is the highest prevalence and the leading cause of morbidity and mortality in women.² WHO shows the prediction of mortality from breast cancer in 2018 with a total number of cases of 2.1 million each year. This makes breast cancer is the leading cause of death in women due to cancer, above from colorectal and pulmonary malignancies.³ In Indonesia, Pathological Based Registration said that breast cancer is the most common malignancy as the number of cases is 18.6%.⁴ Central Java reported breast cancer incidence as many as 3,894 cases (36.84%) in 2005 of all cases of malignancies. In 2005, as many as 759 cases or 19.52% in Semarang of all breast cancer cases in Central Java were found.⁵

Standard therapy, such as surgery, radiotherapy, and chemotherapy, are still the options in cancer treatment.⁶ Surgery and radiotherapy as definitive local therapeutic modalities, during chemotherapy, targeted therapy, and hormonal therapy as systemic therapy modalities.⁷ Combination of chemotherapy is a treatment option that may be administered to patients with breast malignancy.⁸ Doxorubicin, 5-fluorouracil, and cyclophosphamide (FAC) are the most widely used chemotherapy combination. The chemotherapy combination is administered intermittently for 3 - 4 weeks in 6 cycles for 18 - 24 weeks, FAC was administered.⁸ The success rate for chemotherapy is based on the results of the Objective Response Rate after chemotherapy, called Partial Response (PR) and Complete Response (CR). Chemotherapy for breast malignancies has CR / PR only in the range of 20%-40%.⁹ Adjuvant chemotherapy for breast cancer with cyclophosphamide, methotrexate, and 5-Fluorouracil has resulted in abnormal liver function tests and focal abnormalities on radionuclide scans.¹⁰ Liver biopsy specimens have shown severe local inflammation of the liver parenchyma. A study using cyclophosphamide, 5-Fluorouracil, and doxorubicin as adjunctive therapy found 77% of patients had liver function abnormalities that appeared within the
first 3 months after chemotherapy. In the study by Moertel CG et al., there was a dominant increase in alkaline phosphatase levels, as shown by 73% of patients with evidence of hepatotoxicity. This was first observed in the range 1 to 12 months after chemotherapy was started. Treatment with chemotherapy has a beneficial effect on breast cancer prognosis but may cause some side effects such as hepatotoxicity. The mechanism of chemotherapy agents in cancer is various. This chemotherapy produces free radicals that can kill cancer cells as a side mechanism. Free radicals from these byproducts are toxic for cancer cells and healthy body cells, especially actively dividing cells.

A previous study has been directed to use the potential of natural ingredients as agents to reduce side effects such as hepatotoxicity. One of the natural ingredients that have the potential to reduce hepatotoxic effects is honey. Consumption of Honey at a dose of 20 g/day for 2 weeks showed lower levels of alanine aminotransferase and alkaline phosphatase, higher leukocyte and platelet counts versus control subjects in postmenopausal breast cancer patients who did not consume 20 grams of Honey for 2 weeks. There was also increased activity of AST and ALT in the group of experimental animals given cisplatin. The administration of royal jelly honey also inhibits cisplatin-induced hepatic injury, as evidenced by decreasing AST and ALT serum activity. Honey consumption with a dose of 1.2 g/kg dissolved in 250 ml of water for 2 weeks can lower 22% of aspartate transaminase and 18% of alanine transaminase. This study proves that honey can reduce liver enzyme levels.

Based on those mentioned above, this study aims to determine the effect of Honey in preventing hepatotoxicity in ductal invasive breast cancer patients receiving FAC chemotherapy.

METHODS

Research design

This research is an experimental study with a Double-Blind Randomized Pre and Posttest design with a Control Group Design. The research was conducted at the Surgical Oncology Clinic, Chemotherapy Room, and the Dr. Kariadi, Semarang central hospital laboratory during September - December 2020.

Study subjects

In this study, the research subjects (grade III invasive ductal breast cancer patients who met the inclusion criteria) were divided into two groups, the control group and the treatment group, which previously carried out a random allocation.

Sample size determination

The sample in this study were women with invasive ductal breast cancer aged 30-75 years who were treated at Dr. Kariadi Semarang central hospital during the research period. The research subjects were selected by consecutive sampling. Patients who match the study inclusion criteria will be used as research subjects. Women with breast cancer, Invasive ductal histopathological (PA) type, undergoing chemotherapy with FAC cycle I, Good performance status (Karnofsky index 70), age between 30-75 years, Willing to participate in research, sampling was stopped after the number of samples was achieved. The minimum total sample is 18 people in the control group and 18 people in the treatment group.

Intervention and Measurement

Ductal invasive breast cancer patients were divided into 2 groups, the control group who received FAC chemotherapy (n=18) and the treatment group who received chemotherapy and 90 ml/day honey consumption for 14 days (n=18). The patient's AST, ALT, ALP, and GGT enzyme levels before and after 14 days of the study were assessed.

The independent variable in this study was the administration of Honey. The dependent variable in this study was the levels of AST, ALT, ALP, and GGT. Confounding variables are patient age, body mass index, nutrition status, chronic hepatic function disruption, and radiation.

Statistical Analysis

Demographic characteristics data were patient age, Body Mass Index (BMI), education, occupation, Body Surface Area (BSA), and breast cancer stage. Data are presented in tables and graphs. The Shapiro-Wilk normality test was performed before the analysis was carried out because the number of data was less than 50. Analysis of the pre- and post-difference test in the control and treatment groups used Pair T-Test on normally distributed or homogeneous data. In addition, the pre- and post-difference test analysis in the control and treatment groups using the Wilcoxon test in data which is not normally distributed or homogeneous. Analysis between control group delta and treatment group delta was analyzed by Independent T-test if the data were normally distributed or using the Mann-Whitney test if the data were not normally distributed. All analyzes were performed on a computer using SPSS version 15 for Windows. The difference was considered significant if the p-value was ≤ 0.05.

RESULTS

The sample in this study were 18 patients in the treatment group who were given FAC chemotherapy and Honey for 14 days and 18 patients in the control group who received only FAC chemotherapy. There was no significant difference in age, BMI, education levels, occupation, BSA, and ductal invasive (p>0.05) in this study (Table 1).

The results in Table 2 showed a significant decrease in the mean pre-test (50.56±72.85 U/L) and post-test (33.72±44.35 U/L) SGOT enzyme levels in the treatment group (p=0.007). In the control group, it was found that there was a significant increase in the mean pre-test (19.17±7.07 U/L) and post-test (23.22±4.48 U/L) SGOT enzyme levels (p=0.003) (Table 2). However, based on the SGPT assessment, the results showed a non-significant decrease in the mean pre-test (32.28±19.11 U/L) and post-test (29.72±16.96 U/L) SGPT enzyme levels in the treatment group (Table 2).

The results showed no significant increase in the mean pre-test (105.94±77.66 U/L) and post-test (107.00±65.89 U/L) ALP enzyme level in the treatment group (p=0.408) (Table 3). In the control group, it was found that there was a significant increase in the mean pre-test (74.78±31.68 U/L) and post-test (87.22±45.34 U/L) ALP enzyme level in the control group (p=0.031) (Table 3).

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U/L) and post-test (103.17±35.30 U/L) ALP enzyme level (p<0.001) (Table 3). In addition, there was no significant increase in the mean pre-test (54.17±71.20 U/L) and post-test (56.61±58.16 U/L) GGT enzyme level in the treatment group (p=0.102) (Table 3). However, in the control group, there was a significant increase in the mean pre-test (29.06±10.6 U/L) and post-test (51.33±27.50 U/L) ALP enzyme level (p<0.001) (Table 3).

**DISCUSSION**

This study showed a significant difference between the levels of SGOT and SGPT enzymes in the control group and the treatment group. The decrease in SGOT and SGPT enzyme levels in the treatment group indicated an improvement in liver function after 14 days of FAC chemotherapy with Honey. In the control group, there was an increase in the levels of the SGOT and SGPT enzymes. This illustrated that the administration of FAC chemotherapy without Honey gave worse hepatic function from the start even though the increase in enzyme levels was still within normal limits.

The study by Zakaria Z et al., showed that similar results of this study. The study improved the SGOT enzyme and serum albumin levels in grade I-IIIc breast cancer patients after chemotherapy and Honey at a dose of 20 g/day for 12 weeks. Studies showed a significant improvement in control group patients. Zakaria Z et al., stated that the improvement in enzyme levels that occurred in the treatment group was due to the hepatoprotective effect given by honey so that there was no damage to the liver after chemotherapy. This mechanism of the hepatoprotective effect of honey is due to the ability of Honey to reduce free radicals/Reactive Oxidative Stress (ROS) and increase antioxidant defenses, because chemotherapy produces free radicals that can kill cancer cells as a side mechanism. Free radicals of these byproducts are toxic to the cancer cell and healthy body cells, especially actively dividing cells. Phenolic acids and

**Table 1.** Demographic characteristics of patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean±SD)</td>
<td>Treatment</td>
<td>53.56±9.87</td>
</tr>
<tr>
<td>BMI (kg/m²) (mean±SD)</td>
<td>Treatment</td>
<td>24.18±4.83</td>
</tr>
<tr>
<td>Education Levels, n (%)</td>
<td>Treatment</td>
<td>13 (72.20)</td>
</tr>
<tr>
<td>Primary School</td>
<td>Treatment</td>
<td>13 (72.20)</td>
</tr>
<tr>
<td>Junior High School</td>
<td>Treatment</td>
<td>2 (11.10)</td>
</tr>
<tr>
<td>Senior High School</td>
<td>Treatment</td>
<td>3 (16.70)</td>
</tr>
<tr>
<td>College</td>
<td>Treatment</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Occupation, n (%)</td>
<td>Treatment</td>
<td>10 (55.60)</td>
</tr>
<tr>
<td>Work</td>
<td>Treatment</td>
<td>10 (55.60)</td>
</tr>
<tr>
<td>Unemployment</td>
<td>Treatment</td>
<td>8 (44.40)</td>
</tr>
<tr>
<td>BSA (m²) (mean±SD)</td>
<td>Treatment</td>
<td>1.45±0.14</td>
</tr>
<tr>
<td>Ductal Invasive, n (%)</td>
<td>Treatment</td>
<td>13 (72.20)</td>
</tr>
<tr>
<td>Grade II</td>
<td>Treatment</td>
<td>5 (27.80)</td>
</tr>
</tbody>
</table>

*Independent T-Test; Chi-Square; Mann-Whitney U-Test; BMI: Body Mass Index; BSA: Body Surface Area; *Statistically significant if p-value less than 0.05

**Table 2.** Differences in the mean of SGOT and SGPT enzyme levels.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT (U/L) (mean±SD)</td>
<td>Treatment</td>
<td>50.56±72.85</td>
</tr>
<tr>
<td>Pre-Test</td>
<td>Treatment</td>
<td>50.56±72.85</td>
</tr>
<tr>
<td>Post-Test</td>
<td>Treatment</td>
<td>33.72±44.35</td>
</tr>
<tr>
<td>Delta</td>
<td>Treatment</td>
<td>-16.83±29.94</td>
</tr>
<tr>
<td>SGPT (U/L) (mean±SD)</td>
<td>Treatment</td>
<td>32.28±19.11</td>
</tr>
<tr>
<td>Pre-Test</td>
<td>Treatment</td>
<td>32.28±19.11</td>
</tr>
<tr>
<td>Post-Test</td>
<td>Treatment</td>
<td>29.72±16.96</td>
</tr>
<tr>
<td>Delta</td>
<td>Treatment</td>
<td>-2.56±14.37</td>
</tr>
</tbody>
</table>

*Mann-Whitney U-Test; Wilcoxon Test; SGOT: Serum Glutamic Oxaloacetic Transaminase; SGPT: Serum Glutamic Pyruvic Transaminase; *Statistically significant if p-value less than 0.05
flavonoids from Honey are compounds that are responsible for their antioxidant activity. The antioxidant mechanism is known to occur through hydrogen transfer, free radical sequestration, metal ion chelation, flavonoid compounds for hydroxyl & superoxide radical activity. The antioxidant activity enhanced by apoptotic effects has also been observed in hepatocellular malignancy. Administration of Honey with a dose of 1.3 g/kgBW per day showed an increase in antioxidant activity through the induction of the following compounds: glutathione reductase, beta carotene, vitamin C, and uric acid.\textsuperscript{21} Flavonoids have anti-cancer and chemo-preventive properties in various epidemiological studies.\textsuperscript{20}

A previous study said that the increased serum levels of SGOT and SGPT had been associated with the integrity of the hepatic structural damage because these enzyme components are usually located in the cytoplasm of cells and only released into the circulation after liver damage occurs.\textsuperscript{19}

The study showed that administration of royal jelly honey inhibited cisplatin chemotherapy-induced hepatic injury as evidenced by decreased serum SGOT and SGPT activity.\textsuperscript{19} The levels of SGOT and SGPT enzymes are sensitive indicators of liver damage with normal values of SGOT<35 U/L and SGPT<41 U/L.\textsuperscript{22}

In addition, this study also showed an increase in levels of the ALP and GGT enzymes in the control and treatment groups. The increase in the control group was higher than the increase shown in the treatment group and the increase in enzyme levels was still within normal limits.\textsuperscript{23} This indicated that although there was damage to the liver tissue in both groups due to FAC chemotherapy, the subjects who were given honey had a hepatoprotective effect so that hepatic function was not too disturbed, as stated that the increased activity of serum or plasma enzymes was proportional to the level of tissue damage.\textsuperscript{23}

In a study conducted by Moertel CG et al., regarding the use of 5-Fluorouracil and Levamisole as adjuvant therapy, it was found that there was a predominantly increase in alkaline phosphatase levels in 73% of patients, followed by an increase in transaminase and serum bilirubin.\textsuperscript{24} The enzyme elevation was mild and asymptomatic; however, CT scans in some patients showed a fatty liver appearance. This increase was first observed in the range 1 to 12 months after chemotherapy was started and stopped after chemotherapy was no longer given.\textsuperscript{25} As in this study, there was an increase in ALP enzyme levels, but this increase was already seen 14 days after administering FAC chemotherapy.

Vaughan et al. in his study of chemotherapy adjuvant for patients with breast cancer with cyclophosphamide, methotrexate, and 5-Fluorouracil, reported an increase in ALP levels, focal abnormalities on radionuclide scanning, and severe focal inflammation on liver biopsy examination without evidence of metastatic markers, thus emphasizing that this liver damage is not due to metastasis but due to hepatotoxicity of chemotherapy drugs.\textsuperscript{26}

The higher ALP and GGT increases in the control group could be due to the anti-inflammatory effect of Honey acting on the treatment group. Cyclooxygenase-2 (COX-2) in the inflammatory mechanism mediates the metabolic processes of arachidonic acid catalysis to prostaglandins.\textsuperscript{21} In the premalignant and malignant state, COX-2 is overexpressed. The phenolic compounds in Honey are responsible for the anti-inflammatory activity where the mechanism includes stopping the proinflammatory activity of COX-2 & iNOS via phenolic and flavonoids, honey compounds that are known to regulate proteins such as tyrosine kinase, iNOS, ornithine decarboxylase, and COX.\textsuperscript{24,25} Honey also works as an anti-cancer which affects the proliferation of cancer cells, apoptosis of cancer cells, affects the activity of Tumor Necrosis Factor (TNF), and antimutagen.\textsuperscript{21}

In this study, the increase in liver enzymes was associated with administering 5-Fluorouracil chemotherapy, Adriamycin/ doxorubicin, and cyclophosphamide (FAC). The mechanism of toxicity is because doxorubicin and its analogues are metabolized in the liver via microsomal enzymes, and the production of toxic intermediates or immunogens can lead to liver injury. Cyclophosphamide is metabolized entirely by cytochrome P450 in the liver. It can induce sinusoidal obstruction syndrome, which leads to a

**Table 3. Differences in the mean of ALP and GGT enzyme levels.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (U/L) (mean±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Test</td>
<td>105.94±77.66</td>
<td>74.78±31.68</td>
<td>0.113\textsuperscript{a}</td>
</tr>
<tr>
<td>Post-Test</td>
<td>107.00±65.89</td>
<td>103.17±35.30</td>
<td>0.447\textsuperscript{a}</td>
</tr>
<tr>
<td>p</td>
<td>0.408\textsuperscript{a}</td>
<td>&lt;0.001\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>1.06±26.51</td>
<td>28.39±25.56</td>
<td>0.009**</td>
</tr>
<tr>
<td>GGT (U/L) (mean±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Test</td>
<td>54.17±71.20</td>
<td>29.06±10.67</td>
<td>0.154*</td>
</tr>
<tr>
<td>Post-Test</td>
<td>56.61±58.16</td>
<td>51.33±27.50</td>
<td>0.420*</td>
</tr>
<tr>
<td>p</td>
<td>0.102\textsuperscript{a}</td>
<td>&lt;0.001\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>2.44±31.12</td>
<td>22.28±21.97</td>
<td>0.035**</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Mann-Whitney U-Test; \textsuperscript{b}Wilcoxon Test; \textsuperscript{c}Paired Sample T-Test; ALP: Alkaline Phosphatase; GGT: Gamma-Glutamyl Transferase; \textsuperscript{*}Statistically significant if p-value less than 0.05.
direct toxic effect on the cells in the liver sinusoidal. However, further study with bigger sample size as well as prospective approach is recommended to clarify this study limitation.

CONCLUSIONS

Thus, the administration of multiflora honey provides a hepatoprotective effect in invasive ductal breast cancer patients receiving FAC chemotherapy. There was a significant difference in the levels of ALT, AST, ALP, and GGT enzymes in patients with invasive ductal breast cancer who received FAC chemotherapy compared to those who received FAC chemotherapy and Honey.

CONFLICT OF INTEREST

The author declares there is no conflict of interest regarding the publication of the current study.

ETHICAL CLEARANCE

This research was approved and declared ethically feasible by the Ethics Commission of the Public Health Faculty, Universitas Diponegoro, Semarang, with Ethical Clearance number 0201/III/4883/2020.

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AUTHOR CONTRIBUTIONS

All authors equally contribute to the study from the conceptual framework, data gathering, data acquisition, until reporting the study results through publication.

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