The anticancer activity of (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on against DMBA-induced mammary cancer in Sprague Dawley rat through the regulation of microRNA-21 expression


ABSTRACT

Background: The new anticancer is urgently needed due to the high resistance and recurrence of breast cancer. The previous study reported that a new chalcone derivative, (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on, has a potential cytotoxicity against T47D breast cancer cell line. In this study we investigated the anticancer activity of (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on against DMBA-induced mammary cancer in Sprague-Dawley rat and its effect on microRNA-21 expression.

Methods: Twenty-four female rats were divided into six groups. The first group, G1 received corn oil. The groups 2 to 6 (G2, G3, T1, T2, and T3) were induced by DMBA (dissolved in corn oil) 20 mg/kgBW for five weeks. After breast nodule had observed, G2 received the vehicle, and G3 received tamoxifen. Whereas, T1, T2, T3 received (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on with different doses, i.e., 5, 15, and 45 mg/kgBW/day for 21 days, respectively. Tumor size was measured every week for 21 days, and on day 22, plasma and breast tissues were collected to examine the miR-21 expression by qRT-PCR and histopathological feature, respectively.

Result: The result showed that significantly decreased tumor growth (p<0.05) and better histopathological malignant grading in G3, T1, T2, T3 were observed. Moreover, significantly reduced miR-21 relative expression (p<0.05) in G3, T1, and T2 were also observed.

Conclusion: (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on has potential anticancer activity on DMBA-induced mammary cancer in Sprague-Dawley rat through its activity on miR-21 expression. Hence, (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on might be a new anticancer candidate in the future.

INTRODUCTION

Breast cancer is the most prevalent cancer, and the leading cause of death among women in the world.1 Approximately 70% of breast cancer express estrogen receptor alpha-positive (ERα-positive)2,3,4. Hormonal therapy is the most effective therapies for a patient with ERα-positive breast cancer. However, the resistance and recurrence of breast cancer cells to the hormonal therapy is relatively high.3,6 Therefore, the need of new anticancer agents that will be more sensitive is necessary.

Chalcone belongs to flavonoid subgroup7,8 that have been reported to have a wide range of biological activities including as anticancer.9 Previous studies reported that chalcone derivatives had potential cytotoxicity effect against breast cancer cell lines.10,11,12 As part of our program to discover and develop new anticancer, some chalcone derivatives had been successfully synthesized and tested their cytotoxicity against T47D breast cancer cell line. Among the chalcone derivatives tested, (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on was reported to have a potential cytotoxicity against the cancer cell line with an inhibitory concentration 50% (IC50) value 5.28 µg/mL.13 However, the in vivo anticancer activity of this derivative has not been investigated yet. This current study was conducted to investigate the effect of (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on against 7,12-Dimethylbenz(a)anthracene (DMBA)-induced mammary cancer in Sprague-Dawley rat.

Some studies reported that flavonoid affects epigenetic mechanism in cancer treatment. Flavonoid could inhibit DNA methyltransferase and histone deacetylase activities14 as well as regulate microRNA (miRNA) expression to modulate gene expression.15 Most of the breast cancer (90 – 95%) cases are thought to be caused by epigenetic inactivation.3,16 In mammals, miRNAs
are predicted to control protein-coding genes activities more than 60%. Generally, miRNAs have multiple target mRNAs, therefore enhance the possibility to inhibit tumor growth. MicroRNAs can be detected in circulation in a remarkably stable form. It can serve as a potential non-invasive procedure to detect screening biomarker, prognosis, and cancer therapies.

Micro-RNA expression profile in MCF-7 human breast cancer cell line showed that miR-21 was the highest expressed after miRNA microarray analysis of a total of 871 human miRNAs deposited in the miRNA database miRBase. MicroRNA-21 targeted mRNAs that were involved in every hallmark of cancer. The high-level expression of miR-21 was significantly correlated with clinical stage, lymph node metastasis, and shortens survival of the patients, indicating that miR-21 can be used as a prognostic marker for breast cancer and disease progression. This current study also aimed to evaluate the effect of the (e)-1-(4'-aminophenyl)-3-phenylprop-2-en-1-on on plasma miRNA-21 expression of the DMBA-induced mammary cancer in Sprague-Dawley rat.

MATERIAL AND METHODS

Tested compound and Animals. This was an experimental study with the randomized post-test-only control group design. Tested compound, (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on, was synthesized at Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia by Suwito. The chemical structure of (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on is presented in Figure 1.

Female Sprague-Dawley rats aged 3-4 weeks with body weight 32-90 g were obtained from the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada, Yogyakarta. Each animal was housed in an individual cage and maintained at 12 hours light-dark cycle and temperature 24°C at animal house of the Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta and were fed and had accessed to water ad libitum.

The protocol of the study has been approved by Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. Twenty-four female Sprague-Dawley rats were divided into six groups by random allocation. Group G1 (normal) received corn oil, group G2, G3, T1, T2, T3 were induced by 20 mg/kgBW DMBA dissolved in corn oil (Sigma-Aldrich, St Louis) twice a week, for five weeks (modified by Meiyanto et al). Body weight was measured, and the breast was palpated to observe tumor growth every week. After mammary tumor had appeared, group G2 received vehicle (DMSO: Tween 80: saline = 1: 1: 8), and G3 received tamoxifen 6,6 mg/kgBW. Group T1, T2, T3 received intraperitoneal (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on everyday with dosage of 5, 15, and 45 mg/kgBW dissolved in vehicle for 21 days, respectively. Tumor size was measured every week during 21 days. On day 22, plasma samples were collected then the rats were sacrificed by ketamine HCl with a dosage of 0.2 cc/100gBW intramuscularly.

Tumor Growth. Tumor size was measured by caliper on day 0, 7, 14, and 21. In order to prevent observation differences, only one person measured the entire tumor in the study. The formula for tumor volume calculation $V = \frac{1}{2} (\text{Length} \times \text{Width}^2)$. Tumor growth was assessed from tumor volume (cm³) by repeated measures ANOVA analysis using SPSS 17.0 Software.

Histopathological Evaluation. The mammary gland was excised and fixed in 10% PBS-Formalin for 24 hours then replaced with 70% alcohol and processed for histopathological evaluation with hematoxylin-eosin. The mammary tumor was classified as recommended by Goldschmidt and was based on the most pronounced histological pattern observed in more than 50% of the tumor mass.

Tumor Grade. Criteria for the histopathological grade was determined according to Histologic Malignant Grade (HMG) modified by Clemente et al. based on the tubule formation, nuclear pleomorphism, and mitotic rate.

The point of all three features was added together to give a total of the point, called HMG. HMG I, well differentiated (3–5 point); HMG II, moderately differentiated (6–7 point); HMG III, poorly differentiated (8–9 point). First researcher and anatomic pathologist assessed tumor grade (HMG) and histopathological pattern with double-blind
RNA Isolation and Quantitative Real-Time PCR Analysis. Total RNA including microRNA was isolated from plasma according to Qiagen miRNeasy plasma kit (Qiagen, Germany, Cat#217184). It required miRNeasy plasma spike-in control (Cat#219610). Reverse transcription was performed using the Qiagen miScript II RT Kit (Cat#218160). Real-time PCR MyGo Mini (IT-IS Life Science, UK) was carried out using Qiagen miScript SYBR Green PCR Kit (Cat#218073). The primers for PCR were a miScript universal primer and rno-miR-21 specific primer, 5’-TAGCTTATCAGACTGATGTTGA-3’ (IDT, Singapore). All samples were normalized by internal control C.elegans miR-39. The relative expression of miR-21 used livak method (2^\(-\Delta\Delta Ct\text{(miR-21)}\)) and G1 as control. Data were analyzed by one-way ANOVA using SPSS 17.0 Software.

RESULTS

Mammary tumor growth
The result showed that (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on inhibited tumor growth. Tumor growth was lower in rat that received (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on than rat that did not receive (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on as shown on Figure 2. There was a significant main effect of (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on in tumor growth on day 0 and 21 (p<0.05) between DMBA + vehicle group and treatment group that analyzed by repeated measured ANOVA. The significant

Table 1  The relationship between histological grading and type from each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Histopathological type</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2 (DMBA + vehicle)</td>
<td>Tubular</td>
<td>1 (25%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cribriform</td>
<td>0</td>
<td>1 (25%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Comedo Ca</td>
<td>0</td>
<td>0</td>
<td>1 (25%)</td>
</tr>
<tr>
<td></td>
<td>Lipid-rich</td>
<td>0</td>
<td>0</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>G3 (DMBA + tamoxifen)</td>
<td>Tubular</td>
<td>3 (75%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cribriform</td>
<td>0</td>
<td>1 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>T1 (DMBA + chalcone 5 mg)</td>
<td>Tubulopapillary</td>
<td>1 (25%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cystic-papillary</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>T2 (DMBA + Chalcone 15 mg)</td>
<td>Tubular</td>
<td>1 (25%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cribriform</td>
<td>1 (25%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Tubulopapillary</td>
<td>1 (25%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Solid</td>
<td>0</td>
<td>0</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>T3 (DMBA + Chalcone 45 mg)</td>
<td>Tubular</td>
<td>3 (75%)</td>
<td>1 (25%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: G1 (Normal group) showed normal breast without tumor growth.

Figure 2  Mammary tumor growth assessed from tumor volume (cm³) on day 0, 7, 14, and 21. Tumor growth was lower in the treatment group (G3, T1, T2, T3) compared to G2. Group G1: Normal, G2: DMBA + vehicle, G3: DMBA + tamoxifen, T1: DMBA + chalcone 5 mg, T2: DMBA + chalcone 15 mg, T3: DMBA + chalcone 45 mg.

Figure 3  The histopathologic features of treatment groups. A. DMBA + vehicle (grade II). B. DMBA + tamoxifen (grade II). C. DMBA + chalcone 15 mg (grade I). DMBA + chalcone showed more tubular formation and low mitotic count (mitosis: red arrow in insert).
ORIGINAL ARTICLE

Effect of (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on to the tumor growth could be seen from partial eta squared value (0.404). Tumor volume showed significant differences on day 14 between DMBA + vehicle group and DMBA + chalcone dosage 5 mg/kgBW. On day 21, the significant different showed between DMBA + vehicle group and treatment group, i.e. G3, T1, and T2 (p<0.05). Histopathological evaluation

All samples from rat mammary tumor that induced by DMBA were malignant. The most frequent tumor type was carcinoma-simple. The histology malignant grade (HMG) of rat mammary tissue that received (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on were better than group which did not receive (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on (Figure 3). The treatment groups that belonged to HMG I were 75%, and the DMBA + vehicle group was 25%, the other was HMG II and HMG III.

Prognosis of mammary cancer from a combination of histopathological grade and type is more accurate than histopathological type alone. In this study, most of the histopathological type was carcinoma-simple. The histology malignant grade (HMG) of rat mammary tissue that received (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on were better than group which did not receive (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on (Figure 3). The treatment groups that belonged to HMG I were 75%, and the DMBA + vehicle group was 25%, the other was HMG II and HMG III.

MicroRNA-21 expression

(E)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on inhibited tumor growth and repaired histopathological feature were parallel to the reduction level of miR-21 relative expression. The result indicated that miR-21 was overexpressed (5.79±2.24 fold) in DMBA + vehicle group compared to normal rat (p<0.05). Figure 4 showed the relative expression of miR-21, i.e. 1.41±1.13 fold (G3); 0.5±0.1 fold (T1); 2.0±1.8 fold (T2); 3.3±2.1 fold (T3). These result indicated a significantly lower in its expression in treatment groups (G3, T1, and T2) compared to that DMBA + vehicle (G2) (p<0.05). The miR-21 expression was not significant in DMBA + chalcone dosage 45 mg/kgBW (T3) compared to other groups.

DISCUSSION

New chalcone derivatives, (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on was synthesized and evaluated its cytotoxic effect against T47D breast cancer cell line. This compound showed a potent cytotoxic effect with an IC50 value of 5.28 μg/mL. Further study to evaluate the in vivo anticancer activity of the (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on against DMBA-induced mammary cancer in Sprague-Dawley rat was conducted. DMBA is used for an animal model to study estrogen receptor positive-breast cancer.

The result showed this compound could reduce tumor growth and repair histopathology feature (Figure 2 and Table 1). It was demonstrated that the (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on has a potential in vivo anticancer activity. Anticancer activities of some chalcone derivatives against animal cancer models have been reported in previous studies. Loch-Neckel et al. reported in vivo anticancer activity of a hybrid chalcone-quinoxaline compound in a murine xenograft model of U87-MG by a significant reduction of tumors volume. Hayashi et al. reported in vivo anticancer activity of quercetin chalcone against colon-25 tumors implanted in Balb-c mice by a significant reduction in tumor volume.

In the present study, histopathological feature showed a majority of the cancer cells in the DMBA + vehicle group were at a higher grade than the treatment group. It revealed that the most common mammary cancer type was simple carcinoma (85%). Some studies showed the same result. They were reported the most frequently represented cancer type was carcinoma-simple (87%) in rat and (56.8%) in canine mammary cancer.

This compound belongs to flavonoid subgroups that have been reported to exert...
some biological activities, including anticancer.\textsuperscript{9} It was reported that flavonoid affected epigenetic mechanism including microRNA.\textsuperscript{14,15} Flavonoids regulate miRNAs expression through epigenetic modification, transcription factor modulation, and by interfering miRNA maturation process.\textsuperscript{34} In this study, we investigated the miR-21 expression. Overexpression of miR-21 was found in DMBA + vehicle group compared to normal rat (G1 group). Yan et al.\textsuperscript{29} also found up-regulated of miR-21 expression in human breast cancer. In this current study (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on could decrease miR-21 expression. This result is similar to other flavonoid derivatives, 3, 6-dihydroxyflavone that revealed significantly downregulated miR-21 expression and increased miR-34a expression on 1-methyl-1-nitrosourea-induced breast cancer.\textsuperscript{15} Epigallocatechin-3-gallate (EGCG) in green tea was found a significant down-regulation of androgen-regulated miR-21 and up-regulation of miR-330 in tumors of mice treated with EGCG.\textsuperscript{35}

MicroRNA-21 can be used as a prognostic marker for breast cancer and disease progression due to the high-level expression of miR-21 was significantly correlated with clinical stage, lymph node metastasis, and shorter survival of the patients.\textsuperscript{23} A Recent report provided evidence that knock-down of miR-21 increased the sensitivity of ER\textsuperscript{+} breast cancer cell to tamoxifen.\textsuperscript{36} In addition, Wang et al.\textsuperscript{37} reported that miR-21 might play important roles in the formation of chemoresistance of breast cancer cells by targeting PTEN. PTEN is a tumor suppressor gene that antagonizes the phosphatidylinositol 3-kinase (PI3K) pathway to repress tumor suppressor gene that antagonizes the phosphatidylinositol 3-kinase (PI3K) pathway. PTEN is a tumor suppressor gene, which can inhibit the PI3K/Akt pathway, thus leading to cell death and cell cycle arrest. PTEN expression is downregulated in breast cancer, which contributes to the development and progression of breast cancer.\textsuperscript{38} The PI3K/Akt pathway aberration occurs in 70% of breast cancers irrespective of subtype.\textsuperscript{4} Those evidence showed the important roles of miR-21 in cancer therapy.

In this study, the reduction of miR-21 expression had no difference between G2 and T3. Those results showed the effectiveness of chalcone dosage 45 mg/kgBW was lower compared to the chalcone dosage 5 and 15 mg/kgBW. Furthermore, for the next research, it will be better to use a lower dosage that was more efficient to reduce tumor growth and miR-21 expression. It is important to determine the therapeutic windows for this compound to avoid its toxicity.

**CONCLUSION**

In conclusion, (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on has potential in vivo anticancer activities on DMBA-induced mammary cancer in rats as indicated by decreased tumor growth and repairing histopathological grade after treatment with this compound. These activities might be attributed to its effect in decreasing plasma miR-21 expression. Thus, the (e)-1-(4’-aminophenyl)-3-phenylprop-2-en-1-on can be developed as new anticancer in the future.

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**REFERENCES**


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