Anticancer Effect Of Pinostrobin From (Kaempferia Pandurata Roxb) In Benzo(A)Pyrene – Induced Fibrosarcoma In Mice

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Abstract.

Pinostrobin, a flavonoid purified from the Indonesian herb (Kaempferia pandurata Roxb), was tested for antitumor properties in several model system. In vitro, pinostrobin inhibited proliferation of primary human breast cells. human breast cells line MC7, and mieloma cells. The aim of this study was to investigated the inhibitory effect of pinostrobin isolated from Kaempferia pandurata Roxb on the growth of solid tumor of benzo(a)pyrene – induced fibrosarcoma in mice in vivo. The inhibition of solid tumor was measured by weight of tumor and histological observation. These result indicated that pinostrobin showed inhibitory effects on the growth of solid tumor of benzo(a)pyrene – induced fibrosarcoma in mice.

Key Word: Anticancer. Kaempferia pandurata Roxb. pinostrobin. fibrosarcoma.

INTRODUCTION

Kaempferia pandurata (Zingiberaceae) is a plant from Indonesia, known as “temu kunci” in folk medicine, and used as an antitusive, cholic, diuretic, inflammation and disentry (Heyne, 1987). In search for naturally occurring antitumor activity, flavonoid compound as pinostrobin from Kaempferia pandurata rhizome has been done antitumor activities testing vitro on cell culture of primary human breast cancer (Ermawati, 1996) human breast cancer cell line (MC7) (Bail et al., 2000) mieloma cell line (Sukardiman, 2003) The critical biochemical target has been identified. mechanism for the cytotoxicity in cell culture of human breast cancer by inhibition in the activity of DNA Topoisomerase I (Sukardiman et al., 2000) and pinostrobin inhibition aromatase receptor not inhibition estrogen receptor. DNA topoisomerase alter the topological state of DNA. thereby carrying out function essential for several cellular processes including DNA replication, transcription, recombination, DNA repair, and chromosome segregation at mitosis. There are two types of DNA topoisomerase: type I enzymes changes the DNA linking number by transiently breaking one strand of duplex DNA. while type II enzymes transiently breaks both strands (Knut and James, 1992; Cuming and Smyth, 1993; Zahir, 1996).

The in vitro activity is not general correlated with in vivo activity, because the in vivo activity many other factor are influence as metabolism effect from bioactive compound if used oral administration (Constantinu, 1995). In the present study, we investigated the inhibitory effect of pinostrobin isolated from Kaempferia pandurata Roxb on the growth of solid tumor of benzo(a)pyrene – induced fibrosarcoma in mice in vivo.

2. Material and Methods

2.1. Plan material. Kaempferia pandurata rhizoma used in this study were purchased from Ponorogo. East Java. Voucher specimen are deposite at Laboratory of Pharmacognosy. Faculty of Pharmacy. Airlangga University Surabaya.

2.2. General experimental prosedur. Melting point were measured with DTA Analysis. FTIR spectra were recorded on a Jasco FTIR 5300. NMR spectra were recorded on a 138 Tessia Spectrofotometer running at 450 MHz for 13C and 1H. with DMSO as solvents. Chemical shift were measured with tetramethylsilane (TMS) as internal standar. MS spectra measured with a Hewlett Packard.

2.3. Extraction and isolation of pinostrobin. The pulverized rhizome of Kaempferia pandurata (300 g) were extracted at room temperature with n-hexane and the extracts concentrated to dryness under reduced pressure. yielding residues of 47 g. respectively. The residue was subjected to flash column chromatography on silica gel with EtOAc – n Hexane. The compound was recrystallized by hot MeOH. The molecular weight was confirmed by MS. The structure was established by agreement of 1H-NMR and 13C-NMR and data with those of literatur.

2.4. Induced of fibrosarcoma in mice by benzopirena and analysis effect of pinostrobin on inhibit ion growth on solid tumor. Fibrosarcoma were induced in 8 week - old male mice by s.c injection of benzo(a)pyrene from Sigma 0.3 % (b/v) every two days until ten injection. All animal were housed in light and temperature room and fed standart laboratory diet and tap water ad libitum. The solid tumor of fibrosarcoma allowed to grow for 3 month. a size of ~100mm2 and were devided into three groups of three animal each. i.e. the control animal were treated with 0.9% NaCl solution. Thereafter. pinostrobin 0.2 mg/bw / day and 0.4mg/bw/day was injected i.p into the mice on daily basis. At the end 2 and 3 week. the mice were solid tumor. and the tumor were dissected. photographed weighed and histological observation. Histological examination was carried out in all specimen by HE exclusion . benzo(a)pyrene – induced fibrosarcoma were classified into five grades. i.e., a scoring 0 for 0% the growth of solid tumor. a scoring 1 for 25% the growth of solid tumor. a
scoring 2 for 50% the growth of solid tumor. a scoring 3 for 75% the growth of solid tumor. a scoring 4 for 100% the growth of solid tumor (Sakamoto, 1993).

The data of weighed of solid tumor were expressed as:
\[
\text{Inhibition} \% = \left[ 1 - \left( \frac{\text{mean weight of tumor test}}{\text{mean weights of tumor control}} \right) \right] \times 100\% \quad (Yang, 2003).
\]

2.5. Statistical analysis. The statistical significance of difference between group was evaluated by one way anova for data of weighed of solid tumor, and by Kruskal-Wallis rank test for data of histological of solid tumor and \(P < 0.05\) was considered significant (Yang, 2003).

3. Result and Discussion

3.1. Identification of pinostrobin from *Kaempferia pandurata* rhizoma.

![Fig.1 Structural formula of pinostrobin](image)

Pinostrobin crystallized as colorless needles from MeOH, mp 101.8°C. The EIMS showed the molecular ion \([M]^+\) at \(m/z\) 270, corresponding the molecular formula of \(C_{18}H_{14}O_4\). IR and NMR data suggested a fully aromatic flavanone ring system. The \(^1\)H-NMR spectrum displayed signal for one methoxyl group at \(\delta\) 3.76 ppm, five aromatic proton constituen by one singlet at 7.38 ppm, one hydroxyl groups at \(\delta\) 12 ppm. Assignment of the 16 carbon signals of \(^{13}\)C-NMR spectrum.

3.2. Inhibition of growth of solid tumor of benzo(a)pyrene-induced fibrosarcoma by pinostrobin.

3.2.1. The percent change of tumor weight. The solid tumor of fibrosarcoma allowed to growth for 3 month. a size of ~ 100mm\(^3\). Fig. 2 mice with benzo(a)pyrene-induced fibrosarcoma. On this basis, we chose a dose 0.2 mg/bw/day, i.p. the injection were given daily because pinostrobin has short half life. Figure 3 solid tumor of all dose. after were dissected.

![Figure 2. Benzo(a)pyrene-induced fibrosarcoma in mice](image)

![Figure 3. Effect of pinostrobin on growth solid tumor of Benzo(a)pyrene-induced fibrosarcoma in mice](image)

![Figure 4. Histological examination in experimental group](image)

| table 1. Inhibition percent of tumor weight by pinostrobin |
|-----------------|-----------------|-----------------|-----------------|
| Eksperimental groups | Sampel (n) | Meanof tumor weight | inhibition % of growth |
| (1) Control | 3 | 2.3164 ± 1.6126 | 0  |
| (2) Dose 0.2mg/bw/day | 3 | 0.7971± 0.4912 | 62.6  |
| (3) Dose 0.4mg/bw/day | 3 | 0.5373 ± 0.4893 | 76.8  |

\(^a\) vs \(^b\) and \(^a\) vs \(^c\) statistically significant  \((P < 0.05)\)
Table 2. Inhibition percent of growth of solid tumor by pinostrobin with histological examination. ( $Z_{\text{calculated}}$ with average of rank)

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>$R_i1 - R_i2$</th>
<th>$Z_{\text{calculated}}$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control – Dose 0.2mg/bw/day</td>
<td>3.66667</td>
<td>1.33888</td>
<td>0.0918</td>
</tr>
<tr>
<td>Control – Dose 0.4mg/bw/day</td>
<td>5.33333</td>
<td>1.94746</td>
<td>0.0256*</td>
</tr>
<tr>
<td>Dose 0.2mg/bw/day - Dose 0.4mg/bw/day</td>
<td>1.66667</td>
<td>0.60858</td>
<td>0.2709</td>
</tr>
</tbody>
</table>

* : significant with Table Z (Siegel. 1990)

Pinostrobin treatment increase inhibition % growth of solid tumor of benzo(a)pyrene- induced fibrosarcoma in mice. approximately 60 – 80 % of that of control mice ( $P < 0.05$ ) (table 1). Benzo(a)pyrene treatment for 3 months induced fibrosarcoma in mice.

3.2.2. Histology observation. Histological examination was carried out in all specimen by HE exclusion. benzo(a)pyrene – induced fibrosarcoma were classified into five grades. i.e. scoring 0 for 0% the growth of solid tumor. a scoring 1 for 25% the growth of solid tumor. a scoring 2 for 50% the growth of solid tumor. a scoring 3 for 75% the growth of solid tumor. a scoring 4 for 100% the growth of solid tumor. This result show in figure 4.

Pinostrobin treatment increase inhibition % growth of solid tumor of benzo(a)pyrene - induced fibrosarcoma in mice by histological data. approximately 48 – 57 % of that of control mice. and by Kruskal-Wallis rank test was statistically significant between control and dose 0.4 mg/bw/day ( $P < 0.05$ ) (table 2).

4. Conclusion. Pinostrobin was isolated from temu kunci ( Kaempferia pandurata Roxb) shown antitumor effect by inhibits the growth of solid tumor of benzo(a)pyrene – induced fibrosarcoma in mice in vivo.

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REFERENCES