

Antiproliferative activity by ethanolic extract of *Alpinia galanga* Willd in inoculated breast carcinoma cells of C3H mice

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ABSTRACT

Background

The objective of this study is to investigate the anticancer activity of ethanolic extract of *Alpinia galanga* Willd (galangal) using C3H mice breast adenocarcinoma induced by inoculation. In some previous studies ethanolic extract of galangal contains active substances I-Acetoxychavicol acetate (ACA), may be used as anticancer by inhibiting cell proliferation through inhibition of NFκB (Nuclear Factor Kappa B), antiapoptotic and antioxidant effects.

Methods

This experimental study was designed by post test only controlled design group, using 32 C3H mice. The C3H mice were inoculated with tumor cells and then divided into 4 groups: control group (K) and three treatment groups (P1, P2 and P3) given graded doses of ethanolic extract of galangal (225mg/kgBW/day, 450mg/kgBW/day and 675mg/kgBW/day) for 2 weeks. After all mice were terminated, we count the tumor volume of each mice. Correlation between tumor volume and graded doses of ethanolic extract of galangal was analysed by Pearson correlation.

Result

There were no significant differences in tumor volume between control and treatment group at week 1 and 2 but in the end of this study there is a marked differences of tumor volume between groups. Statistical analysis by Pearson correlation showed a significant result.

Conclusion

Oral administration of ethanolic extract of *Alpinia galanga* Willd at the graded doses could inhibit the growth of C3H breast adenocarcinoma by inhibition of cell proliferative activity, that shows by decrease of tumor volume.

Keywords: antiproliferative activity, inhibition of tumor growth, breast adenocarcinoma cells, ethanolic extract of galangal

Background

Breast carcinoma is the second most common cancer in Indonesian women, according to data from the Agency for Cancer Registration, Indonesian Association of Pathologist (IAPI) in 2006. but it is the first common cancer in Padang, West Sumatera. Several treatment modality such as surgery, radiotherapy, hormonal therapy are well known by now but the mortality rate is still high.

Anticancer drug available at present is usually expensive and has many side effects, therefore the patients is looking for alternative therapy such as herbal medicine ¹.

It has been recognized that a large groups of therapeutic agents can stop proliferative activity by many mechanism. Chemopreventive material from plants have prospects as a cancer inhibitor and has fewer side effects. Antitumor and anticancer compounds in plants of which have been widely recognized by the public is *Alpinia galanga* Willd (galangal). Besides used as a food flavoring, galangal is often used as a traditional medicine to treat gastric disorders, eliminate bloating, anti-fungal, eliminate itching, increase appetite, and sore throat ².

Most of the major mediators of tumor growth is a chronic inflammatory, and NF κ B (*Nuclear Factor Kappa B*) is one of the major inflammatory transcription factor that plays a role in the regulation of several tumor cell growth. NF κ B is a therapeutic target of several medicinal plants and also found in tumor tissue. Galangal is one of the herbs that have anti-inflammatory effects and have the inhibitory pathway in NF κ B, thereby reducing the activity of cell proliferation. Galangal also has effect as antiapoptosis and antioxidant. Inhibitor of cell proliferative activity and induction of apoptosis could lead to inhibition of tumor growth, which shows by decrease of tumor volume ^{3,4,5}.

The major phytoconstituents which have been isolated from the galangal is *I-Acetoxychavicol Acetat* (ACA) and hydroxychavicol acetate (HCA). The extracts of galangal showed acetylcholinesterase-inhibitory, platelet-activating factor (PAF)-inhibitory, antimicrobial, antibacterial, antifungal, antioxidant and apoptosis activities. It has been reported that extract of galangal also showed anti-inflammatory, immunostimulating, antinociceptive, hepatoprotective and anticancer activities. Rusmarilin (2003) proved ethyl acetate extracts of galangal inhibit the proliferation of cancer cells in culture using either a groove cancer cells and primary human cancer cells. Ethyl acetate extract of galangal 100 μ g/ml inhibits 100% of lung cancer cell proliferation and at a concentration of 50 μ g/ml inhibits 95% of cell proliferative activity. It is also reported that concentration of 200 μ g/ml could inhibits 76,36% proliferative activity of serous ovarian papillary adenocarcinoma ^{6,7,8}.

Based on the above we are interest to studied the effect of the ethanolic extract of galangal (*Alpinia galanga*) to the activity of cell proliferation that lead to inhibition of tumor growth of breast carcinoma C3H mice with different dose level (225 mg/kg/day, 475 mg/kg day and 675 mg/kg/day).

Methods

This study was an experimental study using post-test only research design controlled design group. The sample used is a strain of C3H mice as much as 32 tails, aged 3-4 months, body weight 15-25 grams were obtained from the Anatomical Pathology Laboratory of the Faculty of Medicine, University of Indonesia. The donor mice of breast adenocarcinoma also obtained from the Anatomical Pathology Laboratory of the Faculty of Medicine, University of Indonesia, experimental mice that widely used for many studies.

Preparation of the galangal extract

The fresh red galangal, 9 months old, that has been harvested, cut into small pieces, dried in an oven 50°C and pulverized to make powder. The dried powder (40 g) was mixed with ethanol (70%), by slowly warming, ethanol has a boiling point of 60 ° C, continuously extracted about 10 times. then evaporated with a *rotary vacuum evaporator* into a *crude* extract. Before oral administration to mice, a crude extract of galangal mixed with aquabidest to make a 0,2 ml solution

Tumor inoculation, administration of ethanolic extract of galangal and measurement of tumor volume

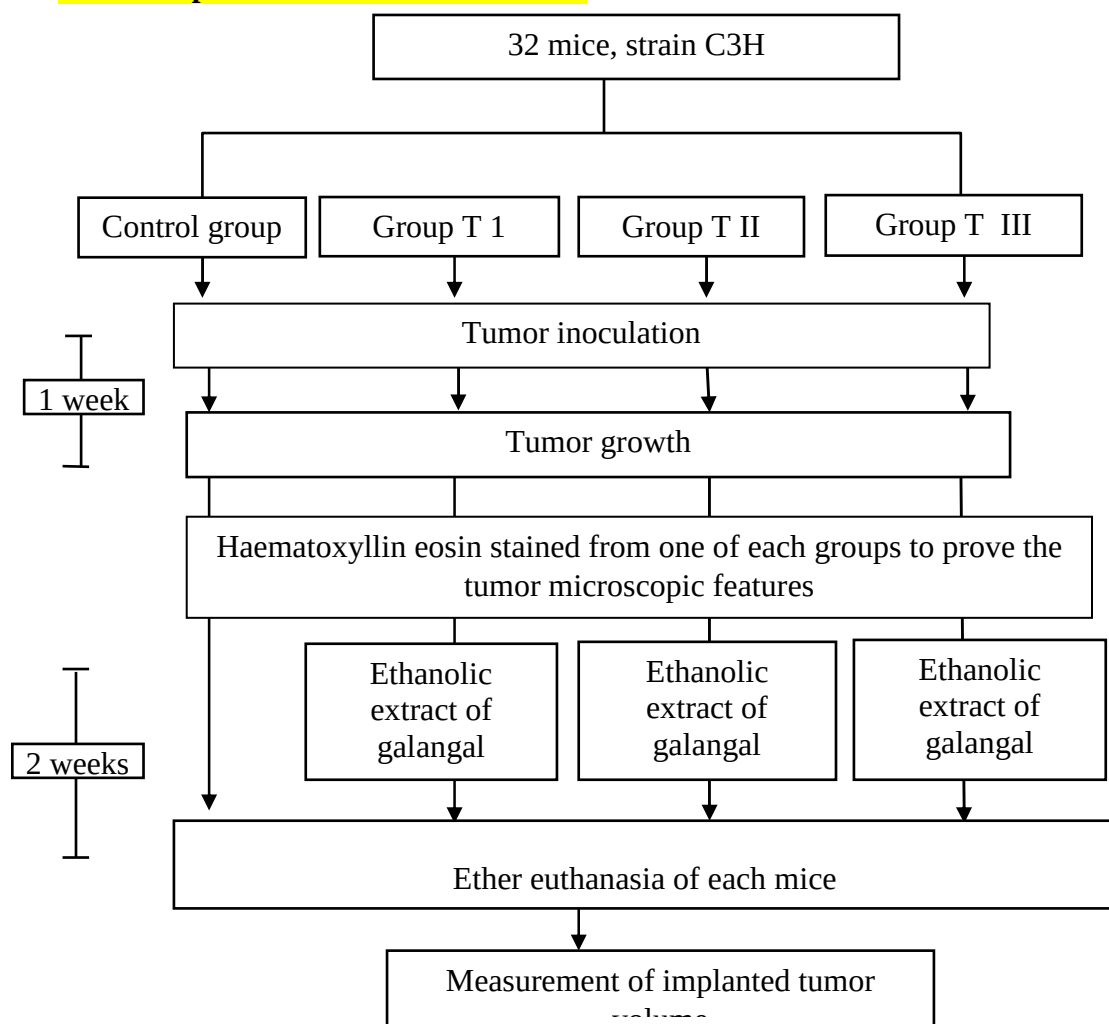
Protocols of current study were approved by the Ethical Committee of Faculty of Medicine, Andalas University. Thirty two strains C3H female mice were divided into 4 groups: control group (K), treatment 1 (P1), Treatment 2 (P2). Treatment 3 (P3), each group consisted of 8 mice C3H. Tumor transplantation was done on day 0 (2 weeks before treatment). The mammary tumor was obtained from donor mice (strain C3H which was also obtained from Laboratory of Anatomy Pathology, Indonesia University. Donor mice was terminated by ether to removed the tumor tissue. After the tumor pulp was made, NaCl solution was added in equal volume to the tumor volume. Tumor inoculation is done by injecting tumor pulp subcutaneously in the right axillar region of recipient mice at a dose of 0.2 ml using insulin syringe, containing approximately 10^6 living tumor cells. After the tumor growth, we measure the tumor volume and terminated one mouse of each groups to make haematoxyllin eosin slide of tumors to prove the microscopic features of tumor cells.

Having proved correct for tumor growth, further mice were treated as follows: Group K were given a standard diet for 2 weeks and did not receive treatment (ethanolic extract of galangal). P1 given a standard diet, and ethanolic extract of galangal received 225 mg/kg/day for 2 weeks. P2 given

a standard diet, ethanolic extract of galangal 450 mg/kg/day for 2 weeks. P3 given a standard diet, ethanolic extract of galangal 675 mg/kg/day for 2 weeks.

Body weight of mice were weighed at the start of the study, subsequent to the first week, the second week of the beginning and the end of the second week before termination. Measurement of tumor volume started 1 week after inoculation, the second week of the beginning and the end of the second week before termination. After the treatment period ended, all the mice were terminated, cut the tumor tissue and measure the tumor volume using the ruler (in mm³)..

Research protocols



Results

Morphological evaluation

At the beginning of the first week after inoculation, the tumor mass has been raised at the site of inoculation. One mouse of each groups were randomly taken and sacrifice to examined tumor cells through histopathological examination with HE staining (*hematoxylin eosin*). From the results of the entire slides obtained positive adenocarcinoma tumor cells (Figure 1).

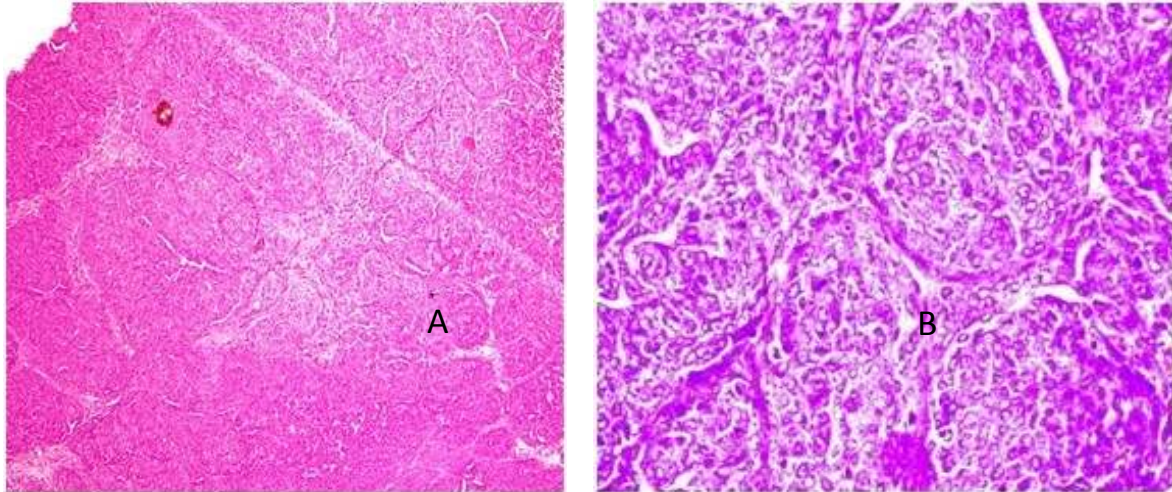


Figure 1. Microscopic features of breast carcinoma of C3H mice (Haematoxyllin eosin).

A. Breast carcinoma, one week after inoculation (Haematoxyllin eosin, 100 x).

B. Breast carcinoma one week after inoculation (Haematoxyllin eosin, 400 x).

Measurement of tumor volume

Before the termination, we measure the body weight of all mice. The weight of the smallest mice prior to final termination is 18,4 g and largest is 25,8 g. After the termination, we cut the tumor and performed the measurement of tumor volume. The smallest mice tumor volume contained in P3 group is 12.6 mm³ and the largest found in the control group which is 216.4 mm³.



Figure 2. Tumor volume of each groups

Table 1. Body weight and tumor volume of each groups

Groups	Body weight (gr)				Tumor volume (gr)		
	H0 (tumor inoculation)	M1	M2	Pre termination	M1	M2	After termination
C 1	22,8	23,9	24	24,1	26,6	49,2	182,5
C 2	22,9	22,7	22,8	22,9	16,6	12,9	192,5
C 3	22,9	23,8	24,1	24,3	19,7	39,5	161,8
C 4	24	25	24,8	22,2	23,8	25,1	187,4
C 5	21,2	22	22,3	21	25,8	25,1	216,4
C 6	20,1	20,5	21	21,9	19,8	23,2	149,7
C 7	21,3	23,1	21,8	24,7	30,1	59,2	142,0
C 8	24	24,4	-	-	22,8	-	-
					Mean = 23,2	Mean = 33,46	
T 1.1	22,3	24,1	24,2	24,7	17,4	15,0	17,1
T 1.2	21,4	21,8	21,5	21,6	20,1	61,8	93,6
T 1.3	24,4	26,1	26,1	26	16,2	35,7	47,8
T 1.4	22,8	22,2	22,3	22,6	17,8	34,1	52,8
T 1.5	20,2	21,3	21,2	21	32,5	48,9	61,3
T 1.6	20,4	22,2	21,8	21,6	15,2	27,7	45,7
T 1.7	22,1	23,5	23,8	23	33,7	33,7	42,4
T 1.8	23,2	24,1	-	-	20,2	-	-
					Mean = 21,84	Mean = 36,7	
T 2.1	20,1	21,6	21,6	20	25,8	60,5	65,4
T 2.2	21,1	20,6	20,5	20,5	20,9	19,9	33,1
T 2.3	22,5	23	23,2	22,3	24,2	32,1	53,6
T 2.4	25,5	24,5	24,7	25,8	27,6	33,8	51,5
T 2.5	20,2	21	21,2	20	29,1	53,9	43,6
T 2.6	20,9	21,2	21,5	20,5	30,9	31,4	63,9
T 2.7	20	21	21	19,6	20,4	34,9	63,9
T 2.8	19,9	21,6	-	-	29,4	-	-

					Mean = 25,56	Mean = 38,07	
T 3.1	20	18	18	19,1	17,6	18,3	54,1
T 3.2	21,2	21,2	21,5	22,2	22,3	19,7	25,8
T 3.3	19,4	17,2	17,2	18,4	18,8	25,3	12,6
T 3.4	20,5	20,5	20,2	20,2	18,9	40,4	71,6
T 3.5	21,2	22	22	21,4	29,4	44,0	42,1
T 3.6	18	18	20,9	21,4	20,8	30,1	59,2
T 3.7	20,5	20,7	18,2	18,5	29,0	31,7	49,0
T 3.8	17	17	-	-	18,4	-	-
					Mean = 22,4	Mean = 29,93	

Table 2. Mean of tumor volume between groups (After termination)

Groups	Mean $\pm$ SD	Median
C	176,04 $\pm$ 26,22	182,500
T 1	51,53 $\pm$ 23,03	47,800
T 2	53,57 $\pm$ 12,08	56,600
T 3	44,91 $\pm$ 20,14	49,000
Pearson correlation ($p < 0,001$)	$p = 0,000$	

From the table 1 shows that the mean tumor volume of the control and treatment groups on week 1 and week 2 is not too different but the mean tumor volume after termination shows marked differences. Statistical analysis by Pearson ($p = 0,000$) shows that there is a significant relationship between the dose of galangal with a reduction in tumor volume.

Discussion

Several studies have shown that a number of herbal medicine have anticancer potential in vitro and in vivo. Those herbal medicine might be good candidate for the development of anti-cancer drugs. Inhibition of cancer cell proliferation, growth factor signaling, induction of apoptosis, etc., contributes to chemopreventive properties⁸.

We used a strain C3H mice in this study because this mice types have a tendency to get a malignancy through genetic susceptibility and transmission of virus MMTV (*Mouse Mammary Tumor Virus*). Using the technique of inoculation in the form of transplant tumor cells will lead to the development of tumor cells. Growth of tumors in mice in the first week after inoculation until the termination varies. In the control group tumor volume tends to increase in size, while in the treatment group tumor volume tends to shrink due to the effects of galangal extract. Turusov and

Mohr (1994) states that the growth of mammary tumors in mice C3H strain could reach 2.4 mm per week.⁹

Table 1 shows the mean of tumor volume for the control and treatment groups. The mean of the highest found in the control group and the lowest in the treatment group 3 (P3). It can be concluded that the ethanolic extract of galangal at a dose of 225mg/kg/day had inhibit proliferative activity, and when the dose was increased up to 675mg/kg/hr, the tumor volume is decreased more smallest.

Galangal (*Alpinia galanga*) with ACA and HCA as active substance, has the potential to reduce the incidence or mortality of cancer by reducing the activity of proliferation and induction of apoptosis. ACA plays a role in apoptosis stimulation by increase the activity of caspase-3, protein that involved in initiation phase of apoptosis. It is also known that ACA can inhibit inflammation processs by inhibiton of nitric oxide (NO) and Cyclooxygenase-2 (COX-2), and elimination of free radicals through strong superoxide anion scavenging activity^{7,10}. Galangal containing ACA also has a role inhibiting NFκB through the inhibition of cell cycle regulatory proteins cyclin D1 which results in barriers to the transition phase of the cell cycle from G1 to S, this activity decreases cell proliferation and tumor cells do not grow. Most of the major mediators of tumor growth is a chronic inflammatory and inflammation process could stimulate the cell to proliferate, inhibit apoptosis and lead to malignant transformation^{3,4,5,10}. He (2009) in his research about *Alpinia oxyphylla* found that this herbal medicine also containt antiangiogenic potential, in vitro and in vivo, by inhibition of neovascularisation¹¹.

Several previous studies have use galangal as an alternative therapy for cancer because galangal has cytotoxic activity. Hartono (2009) which uses ethyl acetate extract of galangal as well as research of Herla Rusmarilin (2003) has been proved that the ethyl acetate extract of galangal was found to inhibit the proliferation of cancer cells in culture good using groove cancer cells and primary human cancer cells.^{6,7,10} Similarly, Lee and Houghton (2005) and Chudiwal et al (2010) also have proved the efficacy of anti-cancer ACA against MCF7 (*Michigan Cancer Foundation 7*) on breast cancer cells and COR L23 in lung cancer.^{7,12}

Conclusion

Oral administration of ethanolic extract of *Alpinia galanga* Willd at the dose 225 mg/kg/day, 450 mg/kg/day and 675 mg/kg/day for 2 weeks after tumor transplant and tumor growth could inhibit

the growth of C3H breast adenocarcinoma by inhibition of cell proliferative activity, that shows by decrease of tumor volume.

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There is no association involvement in sponsorship of the study

Competing Interests :

The authors have declared that no competing interest exist

Authors' Contributions

Author A designed the study with author B, performed the statistical analysis, and wrote the first draft of the manuscript. Author B performed the treatment and literature searches.

Ethical approval

The authors have obtained ethical approval from Ethical Commision, Faculty of Medicine, Andalas University, Padang, Indonesia.

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