ANTICANCER ACTIVITY OF CURCUMA DOMESTICAE RHIZOME EXTRACT AGAINST DMBA-INDUCED COLON CANCER ON MICE

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Abstract

The objective of this research was to examine anticancer activity of curcuma domesticae rhizome extract against DMBA-induced colon cancer on mice. This research was an experimental research using female BALB/C mice aging 2-3 months and 20-30 gBW which were divided into five groups, KNo=normal control group (not induced by DMBA followed by CMC Na 0.5%), KN=negative control group (induced by DMBA followed by CMC Na 0.5%), KP=positive control group (induced by DMBA followed by 11.18 mg/20 gBW capecitabine), D1=first dose group (induced by DMBA followed by 0.482 mg/20 gBW curcuma domesticae rhizome extract), and D2=second dose group (induced by DMBA followed by 1.446 mg/20 gBW curcuma domesticae rhizome extract). The mice will be sacrificed in 30 days after the treatment has been done and the colon will be processed into the paraffin block, then it was stained using haematoxylin eosin staining. The observation were pointed on average of body weight's changes, average of colon weight percentage, and haematoxylin eosin preparation. Statistical analysis that is used were One Way Anova and Krusskal Wallis. The positive control group showed the highest average of body weight's changes (-3.67±3.01) and the lowest one were showed on the normal control group (0.71±1.89). The elevation of average of body weight's changed was only showed on the normal control group (0.71±1.89) and the descendent was showed on dose group (-1.58±1.88), negative control group (-1.79±1.79), second dose group (-2.29±2.93), and positive control group (-3.67±3.01), respectively. The average of colon weight percentage was not interrupted with the treatment statistically (Sig=0.194). The same result was confirmed and achieved macroscopically. The histopathological result showed a real differentiation. Both normal and positive control group showed normal category (score average 1), negative control group showed middle-heavy malignant category (score average 3.86), first dose group showed mild-middle malignant category (score average 2.33), and second dose group showed normal-mild malignant category (score average 1.86). The conclusion of this research was curcuma domesticae rhizome extract has an anticancer activity on dose 0.482 mg/20 gBB and 1.446 mg/20 gBB against DMBA-induced colon cancer on mice.

Keywords : *anticancer activity, Curcuma Domesticae rhizome extract, DMBA, colon cancer.*

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INTRODUCTION

Colon cancer is one of the type of cancer that take place of fourth rank in leading cause of death of cancer types which amounted to 655.000 deaths per year. While in Indonesia from the period 1988 to 1994, colon cancer was ranked the 10th most common cancer occurring in men and women (WHO, 2007). There are several kinds of colon cancer therapy, based on the clinical stage of the disease including surgery, radiation therapy, chemotherapy and immunotherapy. Surgery is the most effective, primarily performed on the disease that was still localized. But when it metastases, the treatment becomes more difficult (Medina & Davis, 2005)

Nowadays, the development of chemotherapy and radiotherapy allow people with advanced clinical stage of the disease or in the case of recurrence for additional therapy (adjuvant). Giving the adjuvant chemotherapy based on fact that patients who appear to have cancer-free after several months or years will haverecurrence or metastases (Sukardja, 2000). It is happened because many patients with colon cancer in Indonesia came already in advanced stage, thus requiring chemotherapy as an adjunct therapy. Although very useful, chemotherapy is a kind of cytotoxic that not only eradicate cancer cells without damaging normal tissue, so the effect of the organ or body system need special attention. Toxicity that often arise in usingchemotherapyare includinghair loss, nausea, vomiting, diarrhea and impaired fertility (Lullman *et al.*, 2000). This is whattoencourage more people to have treatment by using natural substances (Sahu *et al*., 1984).Use of the certain natural substances as medicine arewell known since ancient times based on experience (empirical) and it is derived from generation to generation. But, the verification through bioactivity test (pharmacology), preclinical and clinical test is still not widely practiced in Indonesia (Idris, 2003).

Researches looking for bioactive compounds from plants as anticancer have been made, including turmeric. Curcumin is a compound of the main colour of turmeric and other Curcuma species in addition to demetoxycurcumin and bisdemetoxycurcumin (Stankovic, 2004). Curcumin works as an anticancer by lowering sphingomielinaseacid in colon cancer cells CaCO-2 resulting in barriers to cancer cell proliferation (Cheng *et al*., 2007). Research by Martin-Cordero *et al.* (2007) showed the activity of curcumin as a DNA topoisomerase II poison. DNA topoisomerase II enzyme has an important function inintracellular processeswhichplay a role in the process of replication, transcription, DNA recombination and the proliferation of cancer cells (Hsiang, 1995; Pommier, 1993). The increased expression of this enzyme is reported in human colon cancer cells (Fogt *et al.*, 1997). Mechanism of action of curcumin in colon cancer cells as indicated also by inhibiting prostaglandin production through the barrier activity of lipooxygenase (LOX) resulting on lower product metabolites such as 5(S)-, 8(S)-, 12(S)- and 15(S)- HETE (hydroxycicosatetraenoicacids). This decreased production of LOX metabolites may inhibit the spread, metastases and proliferation of cancer cells (Kawamori*et al*.,1999).

Inhibition of colon cancer cells specifically indicated curcumin through barriers cyclooxygenase-2 (COX-2) expression in human colon cancer cells HT-29 (Goel, 2007). Barriers to the expression of COX-2 occurs as a result of constraints of curcumin on the activity of protein kinase C (Zhang *et al.*, 1999) and NF_KB (Holloway *et al.*, 1998). In colon cancer cells, COX-2 expression showed a marked improvement compared to the normal state (Romano *et al.*, 2003). This overexpression would result in overproduction of prostanoid including prostaglandin which can ultimately lead to various manifestation such as increased cell proliferation (Kinoshita *et al*., 1999), preventing apoptosis (Battum *et al.*, 1998) and accelerating the process of angiogenesis (Tsujii *et al.,* 1998).

The objective of this research was to examine anticancer activity of curcuma

domesticae rhizome extract against DMBA-induced colon cancer on mice.

METHODS

Materials

Curcuma Domesticae Rhizome extract containing curcuminoid 27.58%, DMBA (9,10-dimethyl-1,2-benzanthracene), capecitabine and CMC-Na.

Methods

This research was an experimental research using female BALB/C mice aging 2-3 months and 20-30 gBW which were divided into five groups, KNo=normal control group (not induced by DMBA followed by CMC Na 0.5%), KN=negative control group (induced by DMBA followed by CMC Na 0.5%), KP=positive control group (induced by DMBA followed by 11.18 mg/20 gBW capecitabine), D1=first dose group (induced by DMBA followed by 0.482 mg/20 gBW curcuma domesticae rhizome extract), and D2=second dose group (induced by DMBA followed by 1.446 mg/20 gBW curcuma domesticae rhizome extract). The mice will be sacrificed in 30 days after the treatment has been done and the colon will be processed into the paraffin block, then it was stained using haematoxylin eosin staining. The observation were pointed on average of body weight's changes, average of colon weight percentage, and haematoxylin eosin preparation. Statistical analysis that is used were One Way Anova and Krusskal Wallis.

RESULT AND DISCUSSION

Average of Body Weight's Changes

The result of data analysis in average of body weight's changes during the 30 days period showed that the highest value contained in KP group (-3.67±3.01) and the lowest one found on KNo group (0.71±1.89). The elevation of average of body weight's changed was only showed on KNo group (0.71±1.89) and the descendent was showed on D1 group (-1.58±1.88), KN group (-1.79±1.79), D2 group (-2.29±2.93), and KP group (-3.67±3.01), respectively (Table 1). Statistically, the body weight's changes is affected by the difference in the treatment significantly (Sig=0,035).

Table I. Average of Body Weight's Changes during the 30 Days Test Period

Replication	Groups				
	KNo	KN	KP	D1	D2
1	0	-2	-4	-2	-4
2	-2	1	-4	1	-7
3	3	-3.5	-5	-3.5	-1
4	1	-2.5	-7	-2.5	-1
5	3	0.5	-4	0.5	-1
6	-1	-3	2	-3	-4
7	1	-3	0	0	2
Average	0.71±1.89	-1.79±1.79	-3.67±3.01	-1.58±1.88	-2.29±2.93

Description:
KNo=normal control group (not induced by DMBA followed by CMC Na 0.5%)
KN=negative control group (induced by DMBA followed by CMC Na 0.5%)
KP=positive control group (induced by DMBA followed by 11.18 mg/20 gBW capecitabine)
D1=first dose group (induced by DMBA followed by 0.482 mg/20 gBW curcuma domesticae rhizome extract)
D2=second dose group (induced by DMBA followed by 1.446 mg/20 gBW curcuma domesticae rhizome extract)

Weight loss occurred in all four groups are subjected to the growth of cancerous tissue resulting malnutrition result, so as suggested by Price and Wilson (2000). The occurance of weight loss the most, namely KP group (by 3.67±3.01) also possibly due to the cytotoxic properties of 5-FU (5-fluorouracil) as a result of convension prodrug capecitabine, which attack the high-growth and proliferation tissue of prosterma chemoreceptors stimulation in the area which may cause loss of appetite, nausea and vomiting (Lullman et al., 2000). Weight loss occurred in the D1 group and D2 group in contrast to condition in the other treatment groups for weight loss that occurs not only due to the growth of cancerous tissue, but is also suspected to be due to curcumin activity contained in the extract that lowering adipose tissue through oxidation resistance of LDL (low density lipoprotein) (Asai and Miazawa, 2001).

Average of Colon Weight Percentage

The result of data analysis in average of colon weight percentage during the 30 days period showed that the highest value contained in KNo group (2.16±0.53) and the lowest one found on the D2 group (1.71±0.29) (Table 2). But statistically, the average of colon weight percentage is not affected by the difference in the treatment significantly (Sig=0,194).

However, when pointed from the weight loss occurred with the assumption of the growth of cancerous tissue, it was suspected that the colon cancer's tissue is not a primary cancer. This condition is physically supported by observations that found the cancer in other organs such as stomach, liver and kidney. This is consistent with the result of Milman (1985), Sugiyama *et al*. (2002) and Nicol *et al*. (2004) which states that DMBA is also an inducer of carcinogenesis in the ovary, skin, mammary gland, lung and leukemia. It was indicated that DMBA is not specific to an organ.

Haematoxylin Eosin Preparation

Macroscopic observations of experimental animals showed colonic that there is no real difference between treatment groups (Figure 1). Nonetheless, histopathological observations indicate the presence of a real difference.

Table II. Average of Colon Weight Percentage during the 30 Days Test Period

Replication	Groups				
	KNo	KN	KP	D1	D2
1	1.74	1.97	1.79	2.23	1.43
2	2.11	1.86	1.56	1.56	2.07
3	1.67	1.88	2.77	1.48	1.77
4	2.83	1.93	1.36	1.41	1.48
5	1.88	2.28	1.29	1.51	1.34
6	1.78	2.46	1.85	2.20	2.03
7	3.12	1.97	0	0	1.83
Average	2.16±0.53	2.05±0.23	1.77±0.54	1.73±0.38	1.71±0.29

Description:
KNo=normal control group (not induced by DMBA followed by CMC Na 0.5%),
KN=negative control group (induced by DMBA followed by CMC Na 0.5%)
KP=positive control group (induced by DMBA followed by 11.18 mg/20 gBW capecitabine)
D1=first dose group (induced by DMBA followed by 0.482 mg/20 gBW curcuma domesticae rhizome extract)
D2=second dose group (induced by DMBA followed by 1.446 mg/20 gBW curcuma domesticae rhizome extract)

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Table III. Scoring of Histopathologic Changes in the Colon (Goldsmith, 2008)

Histopathological Changes	Score
Cellular/ nuclear pleumorfism:	
Normal	0
Mild	1
Moderate	2
Marked	3
Hyperchromatic:	
0	0
<20	1
20-29	2
>29	3
Mytosis :	
<2	0
2-9	1
10-19	2
>19	3
<2 = Cathegory 1 (normal) 2-3 = Cathegory 2 (mild malignancy) 4-6 = Cathegory 3 (moderate malignancy) 7-9 = Cathegory 4 (marked malignancy)	

Table IV. Scoring Result of Histopathologic Changes in the Animal's Colon

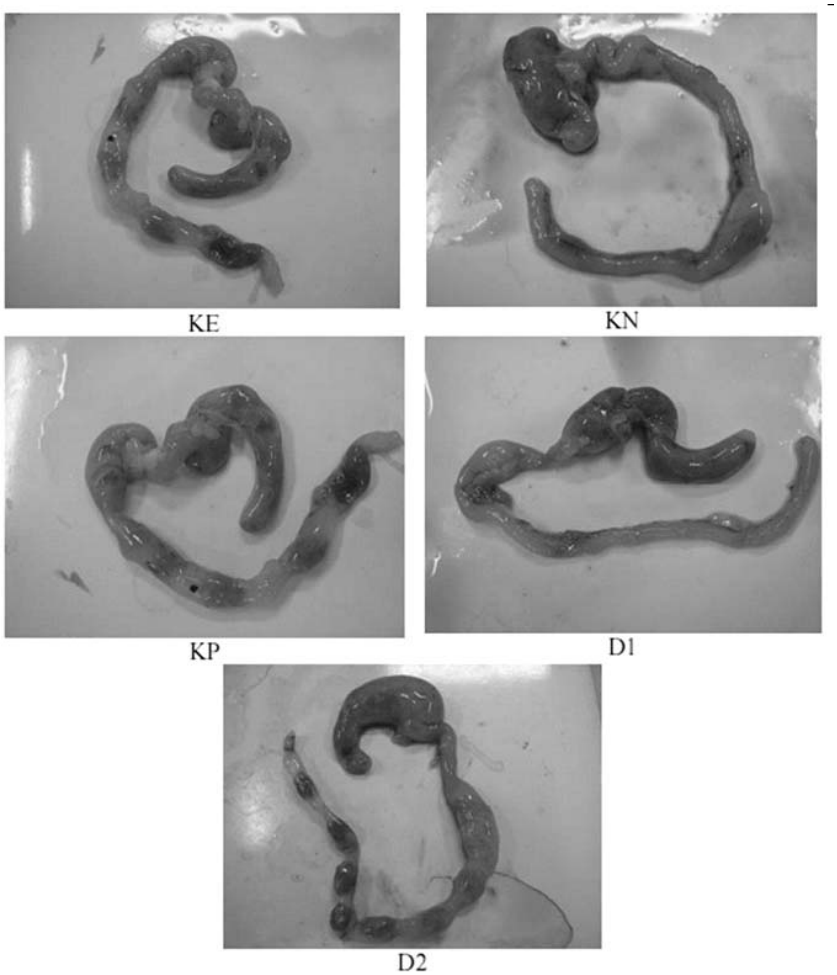
Replication	Groups				
	KNo	KN	KP	D1	D2
1	1	3	1	2	2
2	1	4	1	2	2
3	1	4	1	3	1
4	1	4	1	2	2
5	1	4	1	3	2
6	1	4	1	2	2
7	1	4	0	0	2
Average	1	3.86	1	2.33	1.86

Description:
KNo=normal control group (not induced by DMBA followed by CMC Na 0.5%),
KN=negative control group (induced by DMBA followed by CMC Na 0.5%)
KP=positive control group (induced by DMBA followed by 11.18 mg/20 gBW capecitabine)
D1=first dose group (induced by DMBA followed by 0.482 mg/20 gBW curcuma domesticae rhizome extract)
D2=second dose group (induced by DMBA followed by 1.446 mg/20 gBW curcuma domesticae rhizome extract)

In KNo group and KP group, scoring of histopathologic changes in the colon showed an average score of 1 which means that the growth of cells/ tissues are normal colon; it is different with those that occured in the negative control group, D1 group and D2 group. The KN group showed malignancy category between moderate-severe (mean score 3.86), D1 group

indicates the category of malignancy among mild to moderate (mean score 2,33), whereas D2 group indicates the category of malignancy among normal-mild (average score 1.86). Statistically, the histopathologic changes in the animal's colon is affected by the difference in the treatment significantly (Sig=0). The result showed that the D1 group and D2 group have anticancer activity when they are seen on histophatologic changes that occur, the D2 group showed greater anticancer activity. This corresponds to the amount of extract that its dose is three times larger than D1 group, so activity as an anticancer curcumin becomes larger.

Anticancer mechanism of curcumin is through the mechanism of decreased activity sphingomielinase acid in colon cancer cells CaCO-2 (Cheng *et al*., 2007), as DNA topoisomerase II poison (Martin-Cordero *et al*., 2007), inhibit prostaglandin production through the barier activity of lipooxygenase (LOX) (Kawamori *et al*., 1999) and cyclooxygenase-2 (COX-2) (Goel, 2007).



Description:
KNo=normal control group (not induced by DMBA followed by CMC Na 0.5%),
KN=negative control group (induced by DMBA followed by CMC Na 0.5%)
KP=positive control group (induced by DMBA followed by 1118 mg/20 gBW capecitabine)
D1=first dose group (induced by DMBA followed by 0.482 mg/20 gBW curcuma domesticae rhizome extract)
D2=second dose group (induced by DMBA followed by 1.446 mg/20 gBW curcuma domesticae rhizome extract)

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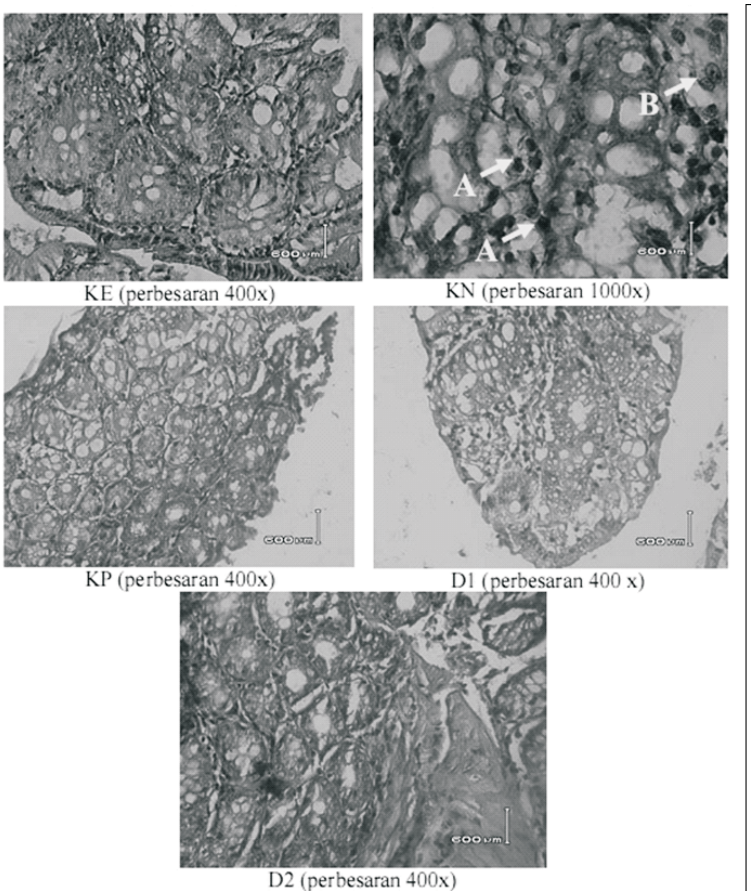

KE (perbesaran 400x) KN (perbesaran 1000x)

KP (perbesaran 400x) D1 (perbesaran 400 x)

D2 (perbesaran 400x)

Description:
A=hyperchromatic
B=mytosis
KNo=normal control group (not induced by DMBA followed by CMC Na 0.5%),
KN=negative control group (induced by DMBA followed by CMC Na 0.5%)
KP=positive control group (induced by DMBA followed by 11.18 mg/20 gBW capecitabine)
D1=first dose group (induced by DMBA followed by 0.482 mg/20 gBW curcuma domesticae rhizome extract)
D2=second dose group (induced by DMBA followed by 1.446 mg/20 gBW curcuma domesticae rhizome extract)

CONCLUSIONS

The conclusion of this research was curcuma domesticae rhizome extract has an anticancer activity on dose 0.482 mg/20 gBB and 1.446 mg/20 gBB against DMBA-induced colon cancer on mice.

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