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RESEARCH ARTICLE

Telomerase and Caspase 3 Expression of Colon Ephitelial Cells Hyperplasia After *Andrographis paniculata* Nees Extract Administration

Ika PN*[1], Imam S[2], Junaidi K[3]

*[1]*Faculty of Medicine, Brawijaya University, Malang, Indonesia.*
[2]*Faculty of Medicine, Airlangga University, Surabaya, Indonesia.*
[3]*Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.*

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ABSTRACT

Incidence and death caused by cancer remains high. The anticancer property of andrographolide has been supported by its ability to induce cell cycle arrest at G0-G1. The aim of this study was to prove telomerase and caspase 3 expression of colon cancer cells after *Andrographis paniculata* Ness extract administration. The female Sprague Dawley rats were treated with oral 7,12-dimethylbenz(a)anthracene (DMBA) 20 mg/kg body weight to induce colon cancer. Twenty five rats were divided into five groups, which were negative control (normal) group, DMBA group, and three groups differed by *A.paniculata's* doses (equivalent to 10 mg andrographolide/kg BW, 30 mg/kg BW, and 100 mg/kg BW). Colon tissues was removed after six weeks treatment. From histologic staining with *haematoxillyn eosin*, it showed that compared to normal group, DMBA induced cell proliferation, nuclear irregularities, and hyperchromatic cells. While the results of immunohystochemistry showed that *Andrographis paniculata* decreased telomerase and increased caspase 3 expresseion of colon epithelial cells hyperplasia. *Andrographis paniculata* extract suppressed cancer cell growth by decreased cell proliferation and increased apoptosis.

KEYWORDS

Telomerase, Caspase 3, Colon, DMBA, *Andrographis paniculata*

INTRODUCTION

In USA, one of four death caused by cancer. In 2010, 102.900 people got colon cancer, while 51.370 of them death.[1] The characteristic of cancer are uncontrolled cell growth, invasive, and metastasis.[2] Colon cancer begins with adenoma that grow progressively and transforms into malignant.[3] Colon cancer therapy consist of surgery, radiotherapy, and chemotherapy.

***Address for Correspondence:**
Ika Putri Nurhayati
Faculty of Medicine,
Brawijaya University,
Malang- Indonesia.
E-Mail Id: kaprinice@gmail.com

One of disadvantage of chemotherapy is it's non selective characteristic, it kills not only cancer cell but also normal cell.[4]

Cancer research paradigm nowdays focused on selectivity of therapy that minimalize its toxic effect on normal cells. Selective therapy acts directly on protein target, growth receptor, or other molecule that excessive expressed in cancer. The understanding of genetic change and cell cycle in cancer cell encourage to find new drug with selective action to cancer cells. Decreasing cells proliferation and increasing apoptosis are two mechanism that is expected from new cancer drug.

Telomerase is a reverse transcriptase enzyme that synthesize telomeric DNA and maintain telomere function.[5] Ninety percent of cancer cell express telomerase activity, whereas normal cell do not express it or express in low amount.[6] Inhibition of telomerase activity can induce apoptosis, a programmed cell death that genetically regulate development and homeostasis.[7,8] There are two pathways to activate apoptosis, extrinsic and intrinsic. Both of them induce effector caspase, caspase 3. Drug that inhibit telomerase activity and induce apoptosis is one of alternative medicine for colon cancer with its selectivity and effectivity properties against cancer cells so that minimize adverse effect.

Sambiloto (*Andrographis paniculata* Ness) consist of diterpenoid, flavonoid, and polifenol as bioactive component and andrographolide as main diterpenoid component. Female Sparegue-Dawley rat used in this study induced with polycyclic aromatic hydrocarbon compound, DMBA. DMBA metabolized by cytochrome p450 enzyme into metagenic epoxide intermediate that form DNA adduct. This DNA adduct cause DNA mutation as carcinogenesis initiation and transform into malignant cells. This study examine effect of *Andrographis paniculata* administration on expression of telomerase enzyme and caspase 3 in colon ephitelial cells hyperplasia in rat induced with DMBA. As hyperplasia is the beginning of cancer development, the sambiloto extract administration may inhibit cancer cells growth.

MATERIALS AND METHOD

Materials used in this study are *Andrographis paniculata* extract obtained from Pharmacognosy and Phytochemistry Department, Faculty of Pharmacy Airlangga University, aquadest, CMC-Na 0,1%, oleum maydis (Mazola), DMBA (Sigma Chemical Co), formaline buffer 10%, chemical ingredient for histopatology preparation and immunohistochemistry, telomerase and caspase 3 antibody (Bioworld).

Sprague-Dawley strain rats (60-200 gram, female, 30-40 days old) were purchased from LPPT-UGM. The animal were kept and fed with same condition. Twenty five rats were randomly divided into five groups, which were negative control (normal) group, DMBA group, and three groups differed by *Andrographis paniculata's* doses (equivalent to 10 mg andrographolide/kg BW, 30 mg/kg BW, and 100 mg/kg BW). Level of andrographolide in extract was measured using Thin Layer Densitometry-Densitometry method. Eluen used are chloroform : methanol = 9:1.

Three treatment groups induced by DMBA with dose 20 mg/kg BW per oral, twice a week for five week. Negative control (normal) grup was given with oleum maydis, whereas DMBA groups was induced by DMBA. After induction, treatment group given with *Andrographis paniculata* extract per oral according to its dose, everyday for six weeks. Extract dissolved with CMC-Na 0.2% in water. Negative control and DMBA group received CMC Na 0.2%. Body weight was measured twice a week. Twenty four hours after the last treatment, colon tissue was removed and kept with neutral buffer formalin solution. Colon tissue then stained using hematoxilin eosin. One slice from each group then examinated using immunehistochemistry method with telomerase and caspase antibody. Method used in immunohistochemical analysis was Labelled Streptavidin Biotin II (LSAB II).

RESULTS AND DISCUSSION

Experimental animal on cancer research is supported by fact that animal have some similiarity with human to response carsinogenic exposure. The similiarity is cancer of ephitelial cells more common than mesodermal one.[9] Long term study using rat as model have beneficial to know cancer stages, such as initiation, promotion, and the progress of carsinogenesis. DMBA used in this study bond with DNA to from DNA adduct that initiate carcinogenesis and caused mutation and permanent damage of DNA. On previous study, DMBA 20 mg/kg BW intragastric can induced 90% tumor mamae and hepar.[10]

Extract used in this study contain of 11.65% andrographolide. Rat induced by DMBA twice a week for five weeks followed by three weeks for maturation. After six week treatment using *Andrographis paniculata* extract, colon tissue was removed and stained with haematoxyllin eosin. DMBA group showed increasing ephitelial cell proliferation as the beginning of cancer but its structure was still well ordered. Cell boundaries become indistinct and overlap each other. Cancer cells absorbs more colors and looks darker than normal cells (hyperchromatic). Nevertheless, cancer cell isn't invasive yet. Other than ephitelial cells, lymphoid tissue also show increased proliferation.

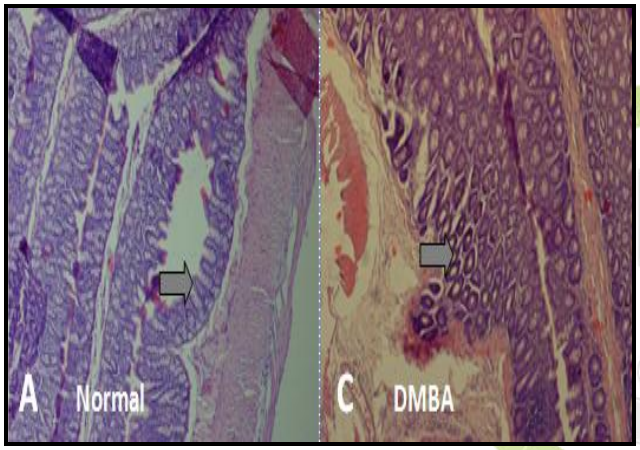

Figure: 1 Haematoxyllin Eosin Staining of Rat Colon. DMBA Group Show Increase Proliferation. Magnification 100x.

Microscopic observation of colon tissue showed that there is morphological change of the cells between negative control group and DMBA group, such as shape, size, and stain. The size of cells that induced by DMBA look bigger than normal and its shape variate include round, oval, and polygonal. Cancer cells also absorbed color more so its nucleus look darker (hypercrhomatin) (Figure: 1). With increasing of dose of *Andrographis paniculata* extract, cell proliferation is decreasing. Intensity of hyperchromatin also is less than DMBA group. Nucleus magnification of treatment groups I (received *A.paniculata* extract equivalent with 10 mg andrographolide/ kg BW) are reduced. Treatment group II show decreasing proliferation and more distinc cell boundaries. Treatment group III also show decreased proliferation, but its difference with group II isn't clear.

Expression of telomerase enzyme can be observed by brown color at nucleus of cancer cells. Control negative group also expressed telomerase enzyme, but it less than DMBA group because in normal condition colon mucosa tissue also doing proliferation. DMBA group expressed telomerase in various intensity. Ratio nucleus and cytoplasm of DMBA group almost one (hypertrophy nuclear). By immunohistochemistry staining using telomerase antibody, DMBA induction increase proliferation of colon epithelial cells (Table:1). Nucleus of cells that expressed telomerase looks bigger and longer. Treatment group that received andrographolide show decrease of telomerase expression (Figure: 2). It means *Andrographis paniculata* extract reduce cells proliferation and limits uncontrolled cells growth as the beginning of cancer. It can be observed from the decrease of brown color of the nucleus.

Table: 1 Number of Cells that Expressed Telomerase (n) Compared with Total Cells (N)

Treatment	**n/N (%)**
Control negative group	0/111 (0%)
DMBA group	69/89 (77.53%)
A.paniculata extract equivalent with 10 mg andrographolide/ kg BW	24/58 (41.40%)
A.paniculata extract equivalent with 30 mg andrographolide/ kg BW	14/44 (31.81%)
A.paniculata extract equivalent with 100 mg andrographolide/ kg BW	12/69 (17.39%)

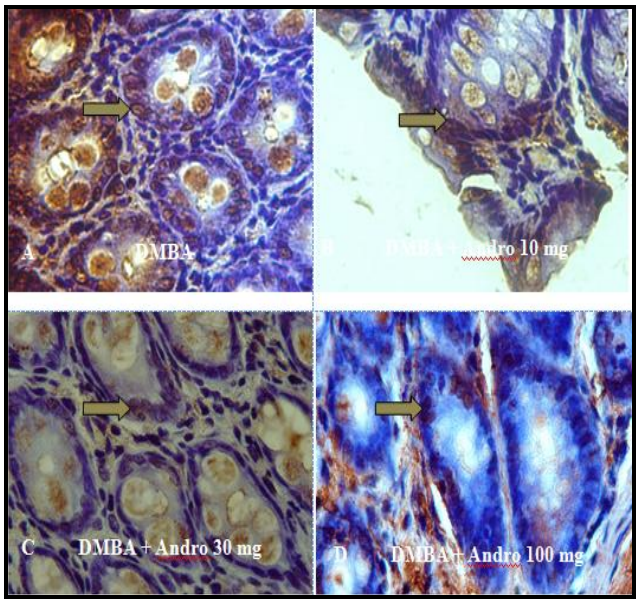

Figure: 2 Immunohistochemistry of Telomerase (Brown Stained). Telomerase was detected in DMBA Group but Expressed Less in Treatment Group. Magnification 1000x

Eighty nine percent of case of bladder, servix, colon, and esophagus preinvasif cancer show very short telomere. It indicate that telomere shortness during cancer growth is a prove of early stage carsinogenesis.[8] DNA adduct bond between DMBA metabolite and DNA cause mutation, especially tumor suppressor gen, p53. p53 contribute to cellular response caused by DNA damage, disturbance and shortness of telomere.[11] Therefore, P53 mutation increase proliferation. As in this study, telomerase also expressed in cancer cells, although yet to be invasive.

Apoptosis, a programmed cell death is one of important mechanism to control cancer cell growth. In this study, apoptosis observed using caspase 3 antibody. Cells that expressed caspase 3 looks brown on its nucleus (Figure: 3). DMBA group show increasing of caspase 3 expression than control negative one. It is because apoptosis is normal way to eliminate cells damage caused by DMBA induction. However, it more increase in treatment group. Color intensity and number of ephitelial cells that expressed caspase 3 of treatment group I and II are more compared to DMBA group. It means *Andrographis paniculata* extract increase cells apoptosis.

Table: 2 Number of Cells That Expressed Telomerase (n) Compared with Total Cells (N)

Treatment	n/N (%)
Control negative group	4/115 (0.34%)
DMBA group	27/118 (22.88%)
A.paniculata extract equivalent with 10 mg andrographolide/ kg BW	22/78 (28.21%)
A.paniculata extract equivalent with 30 mg andrographolide/ kg BW	33/79 (41.77%)
A.paniculata extract equivalent with 100 mg andrographolide/ kg BW	36/70 (51.43%)

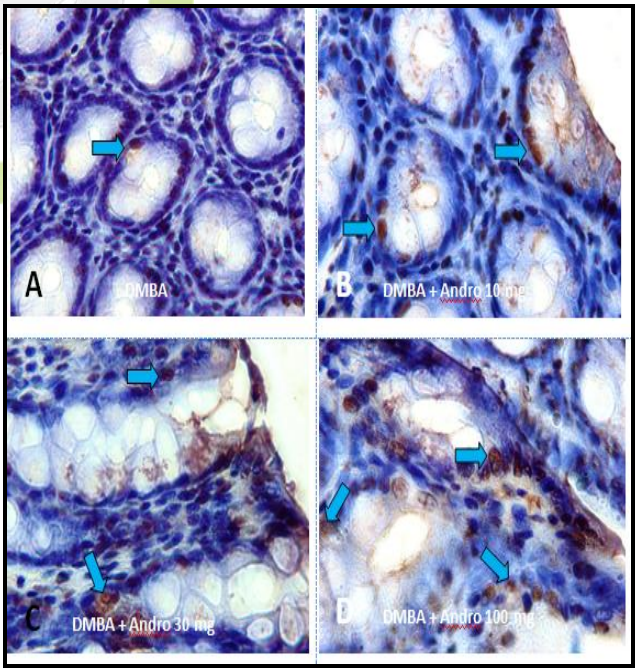

Figure: 3 Immunohistochemistry of Caspase 3 (Brown Stained). Caspase 3 was not detected in DMBA Group but Expressed More in Treatment Group. Magnification 1000x

DMBA metabolite that bound with DNA will trigger DNA reparation and apoptosis. Failure of DNA reparation and apoptosis cause DNA damage become permanent and increase carsinogenasis. This DNA damage can activate oncogene, change apoptosis regulator gen, and inactivate tumor suppressor gen. Color intensity and number of cells that express caspase 3 of treatment group is more than DMBA group. Usage of caspase 3 antibody to detect apoptosis is more sensitive, accurate, and don't depend on DNA fragmentation. Caspase 2 can detect apoptotic cell even before fenotipe change observed.[12] Inhibition to telomerase activity can induce apoptosis.[7,8] Hyperplasia is early stage of cancer development, therefore treatment using andrographis paniculata extract is expected to be alternative therapy to treat colon cancer.

CONCLUSION

Andrographis paniculata extract administration inhibit carsinogenesis of colon ephitelial cells induced by DMBA by decrease telomerase activity therefore decrease cells proliferation and also induce apoptosis characterized with increasing of caspase 3.

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