



Original article

The combination from ethanol extract of moringa leaves (*Moringa oleifera* L.) and ethanol extract of papaya leaves (*Carica papaya* L.) slows the tumor growth in sprague dawley rats induced 7,12-dimethylbenz(a)anthracene[☆]

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ABSTRACT

Objective: This study aimed to see the development of tumor tissue in rats given the combination of ethanol extract of Moringa leaves (*Moringa oleifera* L.) and ethanol extract of papaya leaves (*Carica papaya* L.) as an alternative to increasing nutrition in slowing tumor tissue which will be used for further studies on phytotherapy as an alternative medicine for cancer.

Methods: This study was conducted experimentally using a laboratory Complete Random Design (CRD) method.

Results: The results show that there are different changes in body weight between groups for 12 weeks. However, based on the results of the one way ANOVA test, there are no significant differences between groups ($p > 0.05$). The appearance of tumor tissue was detected at week 7 in the comparison group (DMBA). Whereas in the positive control group and the combination extract group, the solid period began to be felt at the 10th week. Based on the results of histopathological tests in rat organs show that the growth of cancer tissue in all test animals in the treatment group given by DMBA.

Conclusion: The combination from the ethanol extract of Moringa leaves (*Moringa oleifera* L.) and ethanol extract of papaya leaves (*Carica papaya* L.) at a dose of 150 mg/kg BW has activity in slowing the formation of cancer tissue.

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Introduction

Cancer is a disease with a high prevalence, data from GLOBOCAN, International Agency for Research found that breast cancer in the world predicts 18.1 million incidence cancer, outside of non-melanoma skin cancer. There was 9.6 million mortality caused by cancer, outside of nonmelanoma skin cancer.¹

Cancer incidence is not only attacking the elderly, but also productive age and even adolescents also have an increased incidence.²

Various carcinogens also cause the formation of Reactive Oxygen Species (ROS) which can cause the formation of cancer cells during their metabolism, one of which is the DMBA compound

(7,12-dimethylbenz [α] anthracene) which is an immune suppressant compound and organ-specific carcinogen the strong one.³ Currently, cancer therapy applied is radiotherapy, chemotherapy, surgery, and a combination. However, this therapeutic method has not been proven to have a satisfying safety effect and can have severe side effects experienced by patients. Medicinal plants are suspected to have potential as an anticancer with minimal side effects if used with the appropriate dosage and time of use as well as the exact manner of use.^{4,5} One of the plants based on ethnomedicine has been proven to be effective as an anticancer is *Moringa oleifera* L. which has been shown to prevent leukemia, skin papillomagenesis, inhibit the growth of pancreatic cancer cells, and can also be used to reduce the side effects of radiotherapy in bone marrow cancer.^{6,7} In vitro testing proved that *Moringa* leaves can reduce NF- κ B activity in MCF-7 cancer cells. One of the parameters of cancer growth is NF- κ B which functions to stimulate tumor cell cycle progression.⁸ In addition to *Moringa oleifera* L., research shows that the chloroform fraction of papaya leaves have

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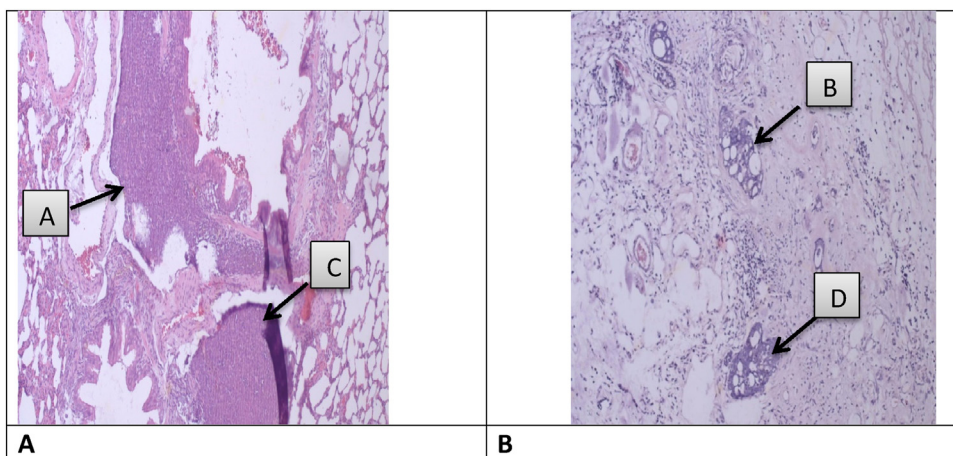


Fig. 1. Histopathological features of tumor tissue after DMBA induction. (A) Histopathology picture of lung organ that has positive cancer (lung carcinoma). (B) Histopathology picture of breast organ that has positive cancer (invasive Ductal Carcinoma).

anticancer activity against myeloma cells. Tests using papaya leaf methanol extract have inhibitory activity against cancer cells.^{9,10}

This study aimed to see the development of tumor tissue in rats given the combination of ethanol extract of Moringa leaves (*Moringa oleifera* L.) and ethanol extract of papaya leaves (*Carica papaya* L.) as an alternative to increasing nutrition in slowing tumor tissue which will be used for further studies on phytotherapy as an alternative medicine for cancer.

Methods

Research location

This study was conducted at the Laboratory of Biochemistry and Molecular Biology, Faculty of Medicine at Hasanuddin University. This study was experimentally using a laboratory Complete Random Design (CRD) method.

Data collection techniques

Before being treated, all test animals need to adjust their physiology, behavior, and nutrition through acclimatization. The acclimatization period is two weeks, adjusting the acclimatization period required by the USDA in the Guideline Stabilization/Acclimation Times for Research Animals (2011), which is a minimum of 7 days. Observations were made on the incidence of cancer, changes in body weight and observation of histopathology test. The process of DMBA production and anatomical histopathological examination is carried out by experienced experts, namely by veterinarians. After week 12, all animals were terminated by cervical dislocation. Then the organs in the animal were taken for histopathology test.

Result

Animal body weight after DMBA induction

After inducing DMBA, the experimental animals were weighed every week to see changes in body weight in each experimental animal. The results showed differences in body weight changes between treatment groups. However, there are no significant differences between groups. Data on changes in body weight of rats after DMBA induction were then processed statistically using the one way ANOVA test method. The test results using the One Way ANOVA method. The result shows that p value > 0.05 which is 0.364

which means the results are not significantly different or not significantly different.

Tumor development

Induction of DMBA was given as much as 1 ml/rats with a dose of 25 mg of DMBA dissolved in 0.5 ml sunflower oil and 0.5 ml NaCl injected subcutaneously in the breast gland region. After induction, observations are made every day to see the development of macroscopic tumor events. Fig. 1 shows a macroscopic observation of tumor mass formation in experimental animals ranging from the induction of DMBA to the extraction of tumor tissue. The picture shows the success of the DMBA induction process which is characterized by the formation of a palpable solid mass in the area around the breast gland, the formation of ulcers in the area around the breast gland and the formation of tumor tissue in experimental animals which are induced by DMBA. Table 1 shows the results of the average measurement of tumor tissue formation based on the time of palpable solid mass (weeks). The table shows that the vitamin C group and the extract combination had a slower mass growth time than the group that was only given DMBA.

Histopathological picture

At week 12, all animals were terminated for the histopathological test. Organs taken from each animal for histopathological test, that is liver, kidney, lung, breast, and digestive tract. Based on histopathological test, Fig. 1 shows a histopathological picture of the development of cancer cells.

Discussion

Based on the results of the study showed that administration from the combination of extracts with a dose of 150 mg/kg BW and 200 mg/kg BW could not provide chemopreventive effects.

Table 1

Results of average measurements of tumor tissue formation based on the time of palpable solid mass appearance (weeks).

Group	Treatment group	Dense mass growth time (weeks)
I	Vitamin C	10.66 ± 0.58
II	Extract 150 mg/kg BW	10.33 ± 0.58
III	Extract 200 mg/kg BW	10 ± 1.16
IV	DMBA	9 ± 1.73

However, the group giving extracts with a dose of 150 mg/kg BW can slow the growth of cancer tissue better. This is based on the results of macroscopic observations which show that tumor formation in the group giving a combination of extracts of 150 mg/kg BW is much slower than another group which not given the extract.

Cancer is a cell disease characterized by disruption or failure of the multiplicative regulatory mechanism and other homeostatic functions in multicellular organisms. Uncontrolled growth of cancer cells is caused by damage to deoxyribose nucleic acid (DNA), thus causing mutations in vital genes that control cell division. Some mutations can turn normal cells into cancer cells. These mutations are caused by chemicals and physical agents called carcinogens. Mutations can occur spontaneously or be inherited.^{11,12}

Exposure to DMBA is able to induce damage to hepatocellular parenchyma causing damage to the liver, tumors, and the risk of malignancy. DMBA is able to be selectively active in the breast, skin, kidney, and liver glands. DMBA is able to induce production of ROS which causes fat peroxidation, DNA damage, and loss of cells that have antioxidant systems.¹³

The ability to inhibit tumor cell formation in the group giving the extract is thought to be due to the presence of chemical compounds in the form of benzyl isothiocyanate and phenethyl isothiocyanate in moringa leaves which has been shown to have anti-cancer activity by inhibiting the activity of NF- κ B in pancreatic and ovarian cancer cell lines. The content of quercetin and kaempferol in moringa leaves, which is included in the flavonoid group that functions as a "scavenging" of free radicals so that ROS is not formed excessively, causing a decrease in NF- κ B activity in MCF-7 cancer cells. In papaya leaves, the content of alkaloids is proven to have anti-cancer activity by inhibiting the activity of the enzyme DNA Topoisomerase. Moringa leaf extract contains chemical compounds benzyl isothiocyanate and phenethyl isothiocyanate which are proven to have anti-cancer activity by inhibiting the activity of NF- κ B in pancreatic and ovarian cancer cell lines. Moringa leaf extract can also reduce the activity of NF- κ B by inhibiting the formation of ROS so that I κ B is not phosphorylated and NF- κ B can be inhibited.¹⁴⁻¹⁶

Recent research shows that the chloroform fraction of papaya leaves has anti-cancer activity against myeloma cells. due to the influence of the chloroform fraction of papaya leaves (*Carica papaya* L.) with the main content of alkaloids allegedly through the initial stages of inhibiting the enzyme DNA Topoisomerase II. With the inhibition of the enzyme activity of DNA Topoisomerase, the bonding process between the enzyme and the DNA of cancer cells is getting longer. So that Protein Linked DNA Breaks (PLDB) will be formed, resulting in fragmentation or damage to cancer cell DNA and subsequently affect the process of replication of cancer cells.¹⁷⁻¹⁹

Conclusion

Based on study results it is known that the combination from the ethanol extract of moringa leaves (*Moringa oleifera* L.) and ethanol extract of papaya leaves (*Carica papaya* L.) at a dose of 150 mg/kg BW has activity in slowing the formation of tumor tissue.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Cancer J Clin* 2018;**68**:394–424.
2. Anwar SL, Raharjo CA, Herviastuti R, Dwianingsih EK, Setyoheriyanto D, Avanti WS, et al. Pathological profiles and clinical management challenges of breast cancer emerging in young women in Indonesia: a hospital-based study. *BMC Womens Health* 2019;**19**:28.
3. Kitakawa D, Cabral LAG, Marques MEA, Salvadori DMF, Ribeiro DA. Medium-term tongue carcinogenesis assays: a comparative study between 4-nitroquinoline 1-oxide (4NQO)-induced rat and dimethylbenzanthracene (DMBA)-induced hamster carcinogenesis. *J Exp Anim Sci* 2006;**43**:219–27.
4. Kostler WJ, Hejna M, Wenzel C, Zielinski CC. Oral mucositis complicating chemotherapy and/or radiotherapy: options for prevention and treatment. *CA Cancer J Clin* 2001;**51**:290–315.
5. Greenwell M, Rahman PK. Medicinal Plants: Their use in anticancer treatment. *Int J Pharm Sci Res* 2015;**6**:4103–12.
6. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: a review on nutritive importance and its medicinal application. *Food Sci Hum Wellness* 2016;**5**:49–56.
7. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in Moringa oleifera leaves protect against chronic disease. *Antioxidants (Basel)* 2017;**6**.
8. Berkovich L, Earon G, Ron I, Rimmon A, Vexler A, Lev-Ari S. Moringa Oleifera aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. *BMC Complement Altern Med* 2013;**13**:212.
9. Fatima U, Shahid S. Pharmacological activities of *Carica papaya* Linn. *J Basic Appl Sci* 2018;**14**:210–6.
10. O'Hare TJ, Williams DJ. *Papaya as a medicinal plant in genetics and genomics of papaya*. New York: Springer; 2014.
11. Taniguchi K, Uchida J, Nishino K, Kumagai T, Okuyama T, Okami J, et al. Quantitative detection of EGFR mutations in circulating tumor DNA derived from lung adenocarcinomas. *Clin Cancer Res* 2011;**17**:7808–15.
12. Boonman ZFHM, Toes REM, Jager MJ. *Tumor immunology in garner and klintworth's pathobiology of ocular disease*. New York: Informa Healthcare; 2008.
13. Paliwal R, Sharma V, Pracheta, Sharma SH. Hepatoprotective and antioxidant potential of moringa oleifera pods against DMBA induced hepatocarcinogenesis in male mice. *J Drug Dev Res* 2011;**3**:128–38.
14. Sinha M, Das DK, Bhattacharjee S, Majumdar S, Dey S. Leaf extract of moringa oleifera prevents ionizing radiation-induced oxidative stress in mice. *J Med Food* 2011;**14**:1167–72.
15. Jeon EJ, Hong SY, Lee JH, Adipokines. Insulin resistance according to characteristics of pregnant women with gestational diabetes mellitus. *Diabetes Metab J* 2017;**41**:457–65.
16. Reda F, Borjac J, Fakhouri R, Usta J. Cytotoxic effect of Moringa oleifera on colon cancer cell lines. *Acta Hort* 2017;**1158**:269–78.
17. Asmah RMA. Proximate Analysis. Antioxidant and anti proliferative activities of different parts of *Carica papaya*. *J Nutr Food Sci* 2014;**4**:2–7.
18. Abdurrochman A, Wulandari RD, Fatimah N. The comparison of classical music relaxation music and the Qur'anic recital: an AEP study. *Reg Symp Biophys Med Phys* 2007.
19. Sankarganesh P, Joseph B, Ganesh Kumar A, Illanjiam S, Srinivasan T. Phytochemical analysis and pharmacognostic value of *Carica papaya* L., Leaf. *J Pure Appl Microbiol* 2018;**12**:751–6.



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