

## The Effect of Mangosteen (*Garcinia Mangostana*) Pericarp Extract on Retinoblastoma Cell Culture Proliferation

Fariz Nufiarwan<sup>1</sup>, Hariwati Moehariadi<sup>1</sup>, Lely Retno Wulandari<sup>1</sup>, Hidayat Sujuti<sup>1</sup>

<sup>1</sup>Departement of Ophthalmology, Universitas Brawijaya, Malang, Indonesia

### ABSTRACT

**Introduction:** To determine the effect of mangosteen (*Garcinia mangostana*) pericarp extracts on retinoblastoma cell culture proliferation.

**Methods:** This study is a true experimental in vitro design with pre and post-test control group design. Research using retinoblastoma cell culture exposed by mangosteen pericarp extract (*Garcinia mangostana*) at a dose of 10 µg/ml, 20 µg/ml, and 40 µg/ml.

**Result:** This study used retinoblastoma cell line cultures and the samples were divided into 4 groups: control group and 3 groups treated with doses of 10 µg/ml, 20 µg/ml and 40 µg/ml and then incubated for 48 hours, and then examined using MTT Cell Proliferation Assay. Group 1 (10 µg/ml) obtained a decrease of 151.8%, group 2 (20 µg/ml) of 134.6% and group 3 (40 µg/ml) of 134.36%.

**Conclusion:** Mangosteen pericarp extract (*Garcinia mangostana*) can reduce retinoblastoma culture cell proliferation.

**Keywords:** mangosteen pericarp extract, cell proliferation, retinoblastoma

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### INTRODUCTION

Intraocular retinoblastoma is the largest primary tumor found in children. In the 20th century, retinoblastoma is a highly fatal disease. Currently, the life expectancy of patients with retinoblastoma about 95% in developing countries. Retino-blastoma case amounts to approximately 4% of all intraocular malignancy in children and about 5000 cases are found worldwide.<sup>1,2</sup>

Signs and symptoms that appear in retinoblastoma depend on the location and spread of the tumor itself. Most often signs are leukokoria (*white papillary reflex*), strabismus and ocular inflammation. Another symptoms that can be occurs is iris heterochromia, spontaneous hyphema, and orbital cellulitis, or inflammation.<sup>1,2</sup>

The aims of retinoblastoma management is to save the lives of patients and maintain the eyeball. Retinoblastoma management include enucleation, exenteration, chemotherapy, laser photocoagulation, cryotherapy, *external-beam radiation* and *plaque radio therapy*.<sup>1,2,3</sup>

Mangosteen is a fruit that grows in the area of Southeast Asia or Indonesia and grow on the Malay Peninsula, Myanmar, Thailand, Cambodia, Vietnam and Malaka. At present it is known that the skin of the mangosteen fruit can prevent the growth of cancer cells. The skin of the mangosteen fruit contains *xanthones* which is a powerful antioxidant, anticarcinogenic, anti-invasive and anti-metastatic.<sup>4,5</sup>

#### \*Correspondence to:

Lely Retno Wulandari,  
 Department of Ophthalmology,  
 Universitas Brawijaya,  
[lelyretnowulandari@gmail.com](mailto:lelyretnowulandari@gmail.com)

Akao *et al* (2008) conducted a study that  $\alpha$ -mangostin have *chemo preventive* effects on short-term rat intestinal carcinogenesis preneoplastik lesions. Castanheiro *et al* (2009) reported on the synthesis and antitumor activity in *pyranoxanthenes* and two *prenylated xanthenes* against cMCF-7 breast adenocarcinoma cells and in 2013, Luo *et al* synthesize a series of *aminoalkoxy substituted benzo [b] xanthone* derivatives and reported antitumor activity *in vitro* on five human tumor cell line. Most of these materials have good tumor inhibition activity.<sup>4,5,6,7</sup>

In this study, mangosteen pericarp extracts with various doses exposed to retinoblastoma culture cells and then observed using *MTT Proliferation Assay* to see the effect of mangosteen pericarp extracts (*Garcinia mangostana*) on retinoblastoma cultured cells proliferation.

**METHODS**

It is true experimental *in vitro* study design with pre and posttest control group design. Research using 24 samples of retinoblastoma cell culture exposed by mangosteen pericarp extract (*Garcinia mangostana*) were divided into 4 groups: control group and three treatment groups. The control group is the group that did not receive any treatment. While the treatment group was given mangosteen pericarp extracts with a dose of 10  $\mu\text{g/ml}$ , 20  $\mu\text{g/ml}$  and 40  $\mu\text{g/ml}$  and incubated for 48 hours then examined using *MTT Cell Proliferation Assay*. This study conducted on October - November 2016 in the laboratory of Physiology, Faculty of Medicine, Brawijaya University and had the fulfillment of the ethical clearance at The Ethical Committee Medical Research in Medical Faculty Brawijaya University.

**RESULTS**

The results is summarized in Table 1. From the table it showed that retinoblastoma cell proliferation decreased in the treated group.

Table 1. Average observations proliferation of retinoblastoma cell cultures in the control group and the group treated with mangosteen (*Garcinia mangostana*) pericarp extract.

Group	N	Mean	Std. Dev	Min	Max
Control	6	221.46	82.25	158.18	335.34
10	6	151.80	15:09	134.84	174.33
20	6	134.66	22.81	105.34	168.92
40	6	134.36	12:01	110.05	140.46
Total	6	160.57	54.83	105.34	335.34

In this study to determine the normality of the data used Shapiro-Wilk method. Based on the analysis showed that

the significant value in the control group and 40 mg / ml  $p < 0.05$  ( $p > 0.05$ ) then the data is not normally distributed.

This study used Lavene test to determine homogeneity. The results of the test is 18.45 with significance of 0.000 ( $p < 0.05$ ) and showed that the data is not homogeneous. Since the data did not normal and homogenous then we used Kruskal – Wallis test. From the Kruskal - Wallis, we obtained significance value of 0.008 ( $p < 0.05$ ), it can be stated there is a difference between the treatment. To find out which treatment group has differences, there should be a post hoc analysis. Tools to conduct a post hoc analysis of Kruskal - Wallis is the Mann-Whitney test. Based on the Mann Whitney test can be seen that there are some differences in the treatment group. It showed that in the control group with 10  $\mu\text{g/ml}$  treatment groups ( $p = 0.037$ ), 20  $\mu\text{g/ml}$  treatment groups ( $p = 0.010$ ) and 40  $\mu\text{g/ml}$  treatment groups ( $p = 0.004$ ) with  $p < 0.05$ . While among the treatment groups 10, 20 and 40 there are no significant differences.

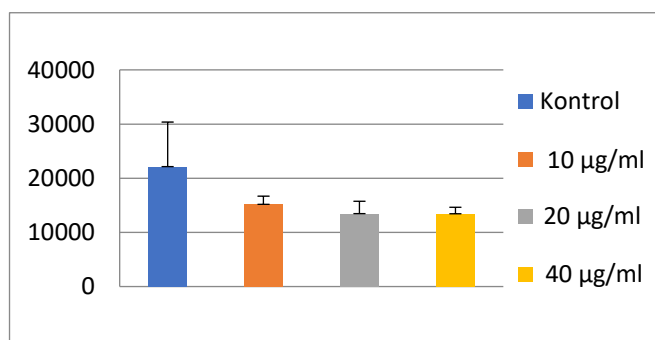


Figure 1. Proliferation charts between groups. The figure shows that the higher doses given the higher the results were obtained.

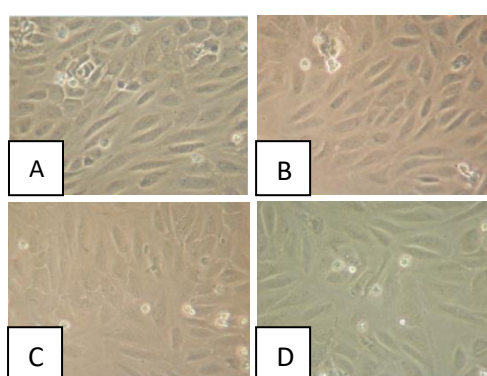


Figure 2. a. Retinoblastoma (RB) culture cell with no treatment (control group), b. RB cell culture with 10  $\mu\text{g/ml}$ , c. RB cell culture with 20  $\mu\text{g/ml}$ , d. RB cell culture with 40  $\mu\text{g/ml}$ .

**DISCUSSION**

Cell proliferation is a process in which the cells multiply to grow and then splitting into two. In normal circumstances turnover and cell renewal will occur as needed through cell proliferation and apoptosis. Tumors arising from excessive proliferation process being offset by

the process of apoptosis. This excessive proliferation can occur due to mutations in the DNA of cells that can cause disturbances in the regulation of cell homeostasis. The imbalance in the cell homeostasis is also known to occur in the retinoblastoma tumor.<sup>3,8</sup>

Xanthenes are compounds derived from the mangosteen pericarp. Pharmacological component of xanthenes has a variety of functions, one of which is as antitumor. As for the antitumor activity xanthenes among other inhibit cell cycle, suppression of tumor cell proliferation, induction of apoptosis and differentiation, reduce inflammation and inhibits adhesion, invasion and metastasis of tumors.<sup>7,9</sup>

The aim of this study to determine the effect of mangosteen pericarp extract (*Garcinia mangostana*) on retinoblastoma cell culture proliferation. Cell culture will be exposed with the mangosteen pericarp extract in different doses of 10 µg/ml, 20 µg/ml, and 40 µg/ml and incubated for 48 hours. Incubation of 48 hours based on previous studies which done by Johnson et al in 2012, carried out in his research that α-mangostin can induce G1 cell cycle arrest intumor cell line prostate cancerat 24 hours and this process lasts up to 48 hours.<sup>10</sup>

There are two methods for calculating the cell proliferation that is direct methods (direct counting) with tryphan blue and MTT assay method. Direct counting is a simple method to assess the integrity of the cell membrane. While basic enzymatic test MTT assay is to measure the ability of living cells based on mitochondrial activity of the cell culture. MTT absorbed into living cells and is broken down into a purple formazan. The concentration of the purple formazan can be measured using a spectrophotometer photometry and proportional to the number of living cells because formazan formed only in the active mitochondria. The greater the absorbance shows a growing number of living cells. In this study, measurement of live cells using method MTT assay because this method has several advantages, relatively more rapid, sensitive, and more accurate when compared to direct counting methods.<sup>11,12</sup>

The result of the calculation of cell proliferation in this study revealed the presentation of live cells based on the results of its absorbance. "Table 1" shows the effect of mangosteen pericarp extract (*Garcinia mangostana*) started at a dose of 10 µg/ml. When compared to the other dose the percentage of viable cell count is more less.

From the data analysis in this study was obtained distribution data is not normal, and a test of homogeneity of variance test data Lavene obtained is not significant indicating variant data is not homogeneous. Because the variant data is not normal and is not homogeneous so to determine differences in the effect of mangosteen pericarp extract (*Garcinia mangostana*) to retinoblastoma cells cultured among the treatment group used a nonparametric test. Non-parametric test used in the analysis of this data is Kruskal Wallis test. Analysis of data using Kruskal Wallis test

showed significant differences between the treatment groups with p value of 0.008 ( $p < 0.05$ ). Furthermore, from the Mann Whitney test results that can be seen in Table 2 states that each treatment group had a significant effect ( $p < 0.05$ ) when compared with the control group. But between treatment group is no significantly different ( $p < 0.05$ ).

Table 2. Table Mann Whitney Test Results.

Treatment Group	Significance
Control with 10	<b>0.037</b>
Control with 20	<b>0.010</b>
Control with 40	<b>0.004</b>
10 with 20	0.200
10 with 40	0.149
20 with 40	0.873

Akao et al in 2008 did the research both invitro and in vivo towards a xanthenes process in colon tumors. In invitro studies Akao explained colon tumor cells cultured with 4 main compound of xanthone which α-mangosteen (αM), β-mangosteen (βM), γ- mangosteen (γM), and methoxy-β-mangosteen (βM-ME) , of the four compounds were used various doses of 10 µg/ml, and 20 µg/ml. The result is after 24 hours of treatment except methoxy-β-mangosteen whole group showed activity inhibits tumor cell growth. But in the group of mangosteen and α-β-mangosteen the dose that can inhibit the growth of tumor cells is a dose of 20 µg/ml, whereas in the group γ- mangosteen dose of 10 µg/ml and 20 µg/ml equally have activity in inhibiting the growth of cancer cells , Li et al in 2014 mentions that, α-mangosteen also affect the growth of prostate tumor cell cultures. They used α-mangostin at a dose of 10 µg/ml, 20 µg/ml and 30 µg/ml. Results from the research showed that all cell cultures obtained inhibit the activity of cell growth with various dose. In cell culture 22Rv1 obtained α-mangostin at a dose of 20 µg/ml and 30 µg/ml significantly inhibited the growth of cell cultures. The greatest inhibit cell growth activity in LNCaP cell cultures at a dose of 30 mg / mL. Wang et al in 2012, also conducts research on the effect of extracts of mangosteen pericarp extract which is use ethanol extract (MPEE) against skin tumor cell cultures SK-MEL-28 (melanoma). They used MPEE with small doses (5 µg/ml) to the largest dose (100 µg/ml). After 48 hours of treatment with a small dose, the anti-proliferation activity against tumor cells occurs. At a dose of 10 µg/ml several cell, loses its shape on the surface and float on the media cell. In the largest doses of 100 µg/ml 25% of the SK-MEL-28 cells dead.<sup>7,13,14</sup>

The limitation of this research is limited in the previous literature review. From some literature also obtained various sources stating differences in dosage and the incubation period. In this study requires research with a

group with a larger sample and the incubation period varied to determine the effective dose and a toxic dose of the mangosteen pericarp extract. Besides, the chemical structure of the mangosteen pericarp extract is not separated specifically due to the lack of available means, the time and the cost is too expensive. Therefore it is necessary in the future to separate of the chemical structures and the effect on normal cell proliferation.

## CONCLUSION

Based on the results and the discussion on this study it can be concluded that the mangosteen pericarp extract (*Garcinia mangostana*) can reduce retinoblastoma cell proliferation. Significant decrease in proliferation obtained between the control group and the treatment group. While between treatment groups were not found significant differences.

## SUGGESTION

Future research using separate active substance from xanthenes ( $\alpha$ -mangostin) to determine the effect directly in retinoblastoma cell culture. It also carried out further research to determine the effective dose and the dose of toxicity from the use of mangosteen pericarp extract (*Garcinia mangostana*) in retinoblastoma cell culture, as well as to determine the effect of mangosteen pericarp extract (*Garcinia mangostana*) in cultures of normal cells.

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