DIPONEGORO MEDICAL JOURNAL
(Jurnal Kedokteran Diponegoro)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
Volume 11, Number 1, January 2022

EFFECT OF *Momordica charantia* FRUIT ETHANOLIC EXTRACT ON MALONDIALDEHYDE (MDA) LEVEL IN BLOOD OF SPRAGUE-DAWLEY RATS INDUCED BY 7,12-DIMETHYLBENZ[A]ANTHRACENE

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ABSTRACT

Background: Cancer is a type of disease with a high rate of diagnostic cases. The real cause of cancer remains unknown. Free radicals can cause cancer by DNA's gen mutation. 7, 12- Dimethylbenz[a]anthracene (DMBA) is often used for researching carcinogenesis. Many types of research have used natural substances as supportive cancer therapies including *Momordica charantia* (bitter melon). Malondialdehyde (MDA) is one of the oxidative damage biomarkers which can significantly increase, especially on breast cancer patients. The success of therapy on a breast cancer patient is shown by decreasing MDA levels in the blood. **Aim:** To investigate the effect of *Momordica charantia* fruit ethanolic extract on malondialdehyde levels in the blood of Sprague Dawley rats induced by 7,12-Dimethylbenz [a] anthracene. **Methods:** This study was a true experimental randomized post-test only with control group design on rats that divided into two groups: X1 given DMBA induction only and X2 given DMBA induction with Momordica charantia fruit ethanolic extract by feeding tube with 200mg/kgbw/day for 14 days. **Results:** The mean of MDA level in group X1=0,83±0,32 and in group X2=0,30±0,19. The result of the normality test of data using the Shapiro-Wilk test obtained abnormal distribution data for one of the groups. The result of independent-samples T-Test showed a significant difference with p=0,001. **Conclusion:** There was an effect of *Momordica charantia* fruit ethanolic extract on MDA level in the blood of Sprague-Dawley induced by 7,12- Dimethylbenz[a]anthracene.
Keywords: *7,12- Dimethylbenz[a]anthracene, cancer, malondialdehyde, Momordica charantia*

INTRODUCTION

Cancer is a type of disease with a high rate of diagnostic cases, including Indonesia. WHO noted that 18 million new cases had been found and diagnosed.[1] Union for International Cancer Control (UICC) stated that there would be an increase in cancer incidence through developing countries, especially Indonesia.[2]

The real cause of cancer remains unknown. Medical personnel has gathered much information about the risk factors of cancer. Cancer itself has so many risk factors that grouped into modified risk factors and non-modified risk factors.[3] One of them includes free radicals. Free radicals are an unstable compound, which can damage the cell first, which becomes oxidative damage that can be accumulated. Therefore, can cause various type of disease by DNA's gen mutation.[4,5]

Polycyclic Aromatic Hydrocarbon (PAH) is a carcinogenic compound generated from hydrocarbon combustion and incomplete fossil fuel burning. The 7,12-dimethylbenze[a]anthracene (DMBA) belongs to the PAH group, and often used for researching, mostly about carcinogenesis.[6] PAH, where is digested in the human body, can be stored inside the adipose tissue. Later will cause lipid peroxidation, which results in cancer development, especially breast cancer.[7]

Many types of research have used natural substances as supportive cancer therapies including *Momordica charantia* (bitter melon). Bitter melon is now also extensively evaluated for its anti-cancer efficacy against various malignancies. It has phenolic and saponin compounds as antioxidants that can be produced with ethanol as extraction solvent.[8] Phenolic & saponin compounds can increase oxygen radical absorbance capacity (ORAC) that can increase antioxidant activity.[9] The compounds are still being studied about the relation toward cancer and oxidative stress. This is categorized into initial therapy that can be evaluated through biochemical's marker. MDA is one of the oxidative damage biomarkers as lipid peroxidation sign in the human body.[10]

MDA has a toxic effect that damages the cell membrane resulting from oxidative stress. If MDA has reached the highest level, then it should be decreased.[11] MDA can be found on every

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cancer, such as colorectal cancer or oral cancer. However, the mammae store more lipids than other places in the human body, resulting in a significant increase in MDA level on breast cancer.[12] The success of therapy on a breast cancer patient is shown by decreasing MDA levels in the blood.[13]

METHOD

This research was conducted at the work station of Examination Laboratory Bioscience Institute of Brawijaya University from April 2020 until June 2020 and was true experimental research with a randomized post-test only control group design, contains 22 female Sprague-Dawley rats from PT. Indo Anilab Bogor that divided into 2 groups with 11 rats each.

Momordica charantia fresh fruit had been sorted to separate with unnecessary materials. After that, fruits were washed and dried with oven on 50°C for few days. Dry fruit now could be crushed by mortar and strained with mesh paper size 40. Next, dry fruit was macerated by put inside erlenmeyer 2,5L with ethanolic alcohol 96% in it for 24 hours with few stirs. Then dry fruit strained by mesh paper, so could collect the filtrate. Repeat the process 3 times with another ethanolic alcohol. After that, filtrate was evaporated by *vacuum rotatory evaporator* and waterbath to collect condensed extract. Finally, extract dilarutkan with aquadest for route per oral with gastric gavage on treatment group.

Sample selection by simple random sampling. The independent variable in this study is the *Momordica charantia* fruit ethanolic extract and the dependent variable in this study is the MDA level in female Sprague-Dawley rats. The rats with the age of 5 weeks old before adaptation, were eligible for this study, and only survived rats after DMBA induction without disability or mortality during the research time, were included to the study. The rats which showed disability or mortality during the research time were excluded from the study. The rats were adapted first for 7 days, then weighed in to determine the dosage for each rat. After the rats had been grouped, every rat was given 60mg/kgbw dosage of DMBA diluted by distilled water through oral using gastric gavage. The next day, the rats were given 200mg/kgbw/day of *Momordica charantia* fruit ethanolic extracts diluted by distilled water through oral using gastric gavage for 14 days. After that, each rat blood was sampled by retro-orbital bleeding technique to be collected for research.

This research using primary data including rat's blood that had been sampled and the rats had reached inclusion criteria during research time. There were two rats in total, which died during research, couldn't reach inclusion criteria. The blood sample had been centrifuged at first to separate blood particles and serum. The blood serum was analysed with the Enzyme-Linked Immunosorbent Assay (ELISA) method by microplate reader to observe the MDA level with a spectrophotometer's wavelength set to 450nm. The MDA level's data was collected with optical density read that was converted into sample concentration value. After the MDA level's data had been collected, we conducted a Shapiro-Wilk test to obtained the normality of data using the computed statistic program. If the test qualifies, we conduct Independent Sample t-test for the hypothetical test. While if the test doesn't qualify, then we conduct the Mann-Whitney U test with a significance *p*-value $\leq$ 0,05. The hypothesis regarding the effect of *Momordica charantia* fruit ethanolic extract on MDA level in the blood of Sprague-Dawley rats induced by DMBA.

RESULTS

Sample collected from Sprague-Dawley rats since April 2020 to May 2020 and obtained 20 rats blood data that met the inclusion and exclusion criteria from a total of 22 rats because the other 2 rats were died during research.

From the table 1, can be seen that the mean of rat's weight in the control group (X1) is 84,50±12,67 and in the treatment group (X2) is 81,30±9,80. The mean of MDA level in the control group (X1) is 0,83±0,32 and in the treatment group (X2) is 0,30±0,19. The p-value in the control group (X1) is 0,018 which obtains abnormal distribution data. While the *p*-value in the treatment group (X2) is 0,203 which obtains normal distribution data. Therefore, we can conclude that the distribution data between the two groups is abnormal. A non-parametric test is conducted for abnormal distribution data.

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Table 1. Result of Mean, Standard Deviation and Shapiro-Wilk Test

Group	Mean±SD	Median (Min-Max)	*p*
Weight (gram)			
X1(n=10)	84,50±12,67	86,50 (67,00-110,00)	
X2(n=10)	81,30±9,80	80,00 (65,00-95,00)	
MDA Level (nmol/ml)			
X1(n=10)	0,83±0,32	0,74 (0,56-1,49)	0,018
X2(n=10)	0,30±0,19	0,24 (0,09-0,61)	0,203

X1: Control group
X2: Treatment group
MDA: Malondialdehyde
P: Probability value

From the table 2, can be seen that the hypothetical test reaches a significance value (*p*-value) of 0,001. Therefore, there was a significant difference between the two groups.

Table 2. Result of Mean, Standard Deviation and Mann-Whitney U Test

	X1	X2	*p*
MDA level	0,83±0,32	0,30±0,19	0,001

X1: Control group
X2: Treatment group
P: Probability value

DISCUSSION

The 7,12-dimethylbenz[a]anthracene compound has cytogenic, mutagenic, and carcinogenic effects.[6] DMBA itself can experience metabolic activity to produce the main carcinogenic named dihydrodiol epoxide.[6] Hence DMBA is more preferred for research in cancer induction, especially breast cancer in rats.[14] Khazaei noted that 60mg/kgbw dosage of DMBA by a single dose can generate breast cancer in less than 5 weeks.[15]

Free radicals can cause damage to DNA due to mutations in genes that can cause changes in DNA structure so that tumor suppression genes are not activated and increased activation of oncogenes can contribute to malignancy.[16] Free radicals have a role in the development of breast cancer by producing malondialdehyde through lipid peroxidation. Free radicals itself becomes a major factor in developing cancer cells in the Hallmark of Cancer theory. The theory is showing of free radicals and nitrite oxide's involvement in the inflammatory process mediated by TNF activation.[17]

Malondialdehyde is generated from lipid peroxidation's process and becomes one of the final products of oxidative stress's biomarkers including superoxide dismutase, catalase, and glutathione peroxidase.[11] The amount of MDA levels is influenced by the amount of free radical levels in the body. Free radicals trigger lipid peroxidase and arachidonic acid production, which initiates DNA damage agents such as MDA and 4-HNE.[18]

The purpose of giving *Momordica charantia* fruit ethanolic extract after DMBA induction is to investigate DNA damage first inside the rat's body which later becomes developing cancer.[6] *Momordica charantia* fruit ethanolic extract has an inhibition effect towards lipid peroxidation due to oxidative stress induction. Carcinogenesis due to DMBA induction can be inhibited by bitter melon plants in the second phase of the enzyme detoxification pathway.[19]

Bitter melon plants have been used widely as traditional herbal therapy for curing various diseases, including cancer.[8] The ethanolic extract was recorded to have the antioxidant activity of IC_{50} from 0,05009mg/ml to 0,05688mg/ml.[20] Concerning antioxidants, phenolic content inside the fruit ethanolic extract can reduce free radical activity, by increasing antioxidant activity by activating antioxidant enzyme. Oxygen radical absorbance capacity (ORAC) is one of the factors that can influence antioxidant activity. Phenolic compounds can enhance ORAC value with positive correlation, so antioxidant activity can be increased.[9] Furthermore, the activation produces glutathione peroxidase and superoxide dismutase.[21]

The increasing of antioxidant enzyme activity leads to enhance the antioxidant's barrier system itself in case of detoxifying free radicals (reactive oxygen species).[22] This study proves that *Momordica charantia* fruit ethanolic extract has an effect on decreasing the MDA level in the rat's blood. It can help prevent cancer cell growth, by acting as an antioxidant in fighting free radicals. The ethanol extract of *Momordica charantia* fruit has a protective effect in the body with the help of antioxidant enzyme activation, reduces fat peroxidase and decreases the expression of proinflammatory cytokines.[23]

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CONCLUSION

The researcher can conclude that there was an effect of *Momordica charantia* fruit ethanolic extract on MDA level in the blood of Sprague-Dawley induced by 7,12-Dimethylbenz[a]anthracene. Further studies and researches are required to investigate various dosages of the ethanolic extract itself on the variety of effects, and measuring the MDA level before and after DMBA induction to compare the effects between these two events as well.

Ethical Approval

This research protocol has received ethical clearance from the Medical and Health Research Ethics Commission (KEPK), Faculty of Medicine, Diponegoro University / Dr. Kariadi Hospital, Semarang with letter number No. 53/EC/H/KEPK/FK-UNDIP/VI/2020.

Conflicts of Interest

The authors declare no conflict of interest

Funding

No specific funding was provided for this article.

Author Contributions

Writing-original draft preparation, Ongky Surya Wijaya Hendro; Writing-review and editing, dr. Ari Budi Himawan, M.Kes(Epid.).

Acknowledgments

This work was supported by Department of Surgery & Department of Public Health, Faculty of Medicine, Diponegoro University.

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