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(RESEARCH ARTICLE)

Antitumor activity of protein fraction of *Jatropha curcas* In DMBA and UV B induced skin tumors in mice: Expression of Bcl-2

Hanif Nasiatul Baroroh * and Warsinah

Department of Pharmacy, Faculty of Health Sciences, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia.

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Abstract

UV radiation and 7,12-dimethylbenz[α]anthracene (DMBA) induced skin tumors can be seen from the decreased Bcl-2 as a suppressor gene regulating programmed cell death and apoptosis. This study aims to determine the antitumor activity of protein fractions from *Jatropha curcas* leaves against skin tumor cells through the expression of Bcl-2 protein in mice-induced DMBA and UVB. This study used 40 mice divided into 4 groups. Group 1 was the control group without treatment. Group 2 was induced 0.2% DMBA (200µl/mice) and UVB (5x/week) for 8 weeks. Groups 3 and 4 were induced 0.2% DMBA (200µl/mice) and UVB (5x/week) for 8 weeks of continued treatment with DMSO, and the protein fraction dose of 2 mg/0,1 ml for 4 weeks, respectively. Immunohistochemistry examination from the tissue skin of mice was performed by using Bcl-2 monoclonal antibody on week 8 (after induced UVB) and on week 12 (after treatment). The results showed that Bcl-2 expression was significantly different in group 2 (induced DMBA and UVB) and group 4 (protein fraction) ($p<0,05$). Expression of Bcl-2 was found to be lowest in the group with treatment by protein fraction dose of 2 mg/0,1 ml. The highest expression was found in the group with induced DMBA and UVB. Decreased expression of Bcl-2 is a sign of apoptosis, so the protein *Jatropha curcas* has antitumor through induction of apoptosis.

Keywords: *Jatropha curcas*; Skin tumor; Bcl-2; Apoptosis

1. Introduction

Tumor is a disease that requires serious attention because tumor is a major public health problem in the United States and developing countries. The incidence of skin tumors in the United States could lead to the death of one in four people [1]. In Indonesia, skin tumors are increasing by following per under with increasing age. The increase in exposure dose of ultraviolet (UV) B can increase the risk of skin tumors. Administration of 7,12-dimethyl benz [α] anthracene (DMBA) can cause tumors in mice [2]. UVB and DMBA were responsible for causing the formation of Bcl-2 mutations in cases of skin tumors [3] [4].

Apoptosis is defined as programmed cell death. This process is essential in the homeostasis of cell numbers in organs to determine the cell numbers of the whole organism. Defects in apoptosis or apoptotic prevention can result in tumors that are caused or enhanced by excessive apoptosis [5]. The apoptotic signal involves the activation of several powerful enzymes (mainly the Bcl and Caspase families). The Bcl family has a controlling role in maintaining the apoptotic signal. BcI-2 protein expression is thought to result in the accumulation of cells by inhibition of apoptosis. The bcl-2 gene (also known as BCL2) encodes for a mitochondrial protein thought to prevent apoptosis of normal cells. Bcl-2 gene rearrangement and overexpression of BcI-2 protein have been found in lymphoma suggesting the existence of several molecular mechanisms for Bcl-2 protein overexpression. The Bcl2 gene is overexpressed in most cell lines, as opposed to other histological tumor tissues a fact that has led to the hypothesis that it could be implicated in the pathogenesis of this tumor [6].

* Corresponding author: Hanif Nasiatul Baroroh

Tumor therapy is chemotherapy and surgery. Chemotherapy is less selective because normal cells can be damaged. Herbal medicine as chemopreventive can be used as an alternative therapy. Herbal products from medicinal plants are preferred because of higher safety, efficiency, cultural acceptability, and lesser side effects.

One of the traditional medicines used by the people of Indonesia is *Jatropha curcas* leaves. Saponin, polyphenols, and flavonoids are compounds that are verified to be found in *Jatropha curcas* leaves. Compounds that have been isolated from *Jatropha curcas* leaves include the flavonoids apigenin and its glycosides vitexin and isovitexin, the sterols stigmasterol, 3 -D-sitosterol and its 3 -D-glucoside[7]. This plant is traditionally used in various diseases like ulcers, mouthwash, edema, rheumatic, and pain. Besides seed oil is used as a cure for edema, ointment, rheumatism, and skin diseases [8]. The previous investigations indicated that the RIP gene of curcin was cloned from *Jatropha curcas* seeds and the antitumor activity of curcin was demonstrated [9][10]. The roots, stems and leaves of *J.curcas* had cytotoxic activity on human colon adenocarcinoma and human hepatocytes [11]. Protein fraction from *J.curcas* leaves had cleaving activity on supercoiled DNA, which was indicated by the enzymatic activity of ribosome-inactivating proteins (RIPs) which have potential as antitumors [12]. We investigated the antiproliferation activity of *Jatropha curcas* of the expression of *bcl-2* (B-cell leukemia/lymphoma-2) in skin tumors in mice by immunohistochemical procedures.

2. Material and methods

2.1 Materials

The Jatropha leaves were collected from the Faculty of Biology, Jenderal Soedirman University, and were authenticated in the Department of Plant Taxonomy, Jenderal Soedirman University. Healthy female Balb/c mice (19-22 g body weight) were obtained from the Faculty of Medicine, Universitas Gadjah Mada. The animals were kept in plastic cages under hygienic conditions and were provided standard animal feed. Before initiating the experiment, the animals were adapted to the laboratory conditions for a week.

2.2 Methods

2.2.1 Preparation of protein fraction from Jatropha curcas leaves

Preparation of *J.curcas* protein extract from leaves was prepared by grinding in 0.14 M sodium chloride in 5 mM sodium phosphate buffer pH 7.2. The extract was centrifuged (15,000 rpm, 15 min). The supernatant (crude extract) was separated from the sediment, followed by protein precipitation using acetone 1:1 and centrifuged. Then the precipitated proteins were obtained by separation from the supernatant, called protein fraction.

2.2.2 Study of protein fraction on in vivo model of skin cancer

Forty female mice were divided into 4 groups for the present study. Group I animals served as normal controls. Group II animals received topical application of DMBA 0,2% in acetone (200µl) followed by UVB UV B 0,4 J/cm^2 5x weekly for 8 weeks. Group III animals received topical application of DMBA 0,2% in acetone (200µl) followed by UVB UV B 0,4 J/cm^2 5x weekly for 8 weeks and DMSO, and group IV animals received topical application of DMBA 0,2% in acetone (200µl) and followed by UVB UV B 0,4 J/cm^2 5x weekly for 8 weeks and *J.curcas* 2 mg/ml. DMBA was applied topically on the depilated back of mice before exposure to UVB. DMSO and *J.curcas* were applied topically. At week 8 and the end of the experimental period (week 12), full-grown tumors were selected for the present studies.

2.2.3 Histopathological studies

Tumors, normal and treated skin removed from sacrificed mice were immediately fixed in 10% formalin fixative for 24h. The tissues were then dehydrated in an ascending series of alcohol and kept in a mixture of absolute alcohol (1:1). Finally, tissue pieces were embedded in paraffin wax and 7-micron thick sections were cut and spread on glass slides, stained with hematoxylin and eosin, and viewed under light microscope and photographed.

2.2.4 Immunohistochemistry

Immunohistochemistry was carried out on 5 µm tissue sections from paraffin blocks using the avidin-biotin immunoperoxidase method, The following antibodies were used: Rabbit anti-human multiclonal BCL-2 antibody. Briefly, the paraffin sections were deparaffinized with xylene and rehydrated through a series of descending-graded ethanol. Endogenous peroxidase activity was blocked by incubation for 15 min in 0.3% H_2O_2 buffer. To unmask the epitopes of BCL- microwave-processing pretreatment was carried out in a citrate buffer, pH = 6.0 for 10 min. Subsequently, Rabbit anti-human multiclonal BCL-2 antibody was applied. Biotinylated secondary antibody and avidin-biotin-complex with horseradish peroxidase were applied, followed by the addition of the chromogen. Finally, slides

were counterstained with hematoxylin, dehydrated in ascending ethanol, cleared with xylene, and mounted with coverslips using a permanent mounting medium.

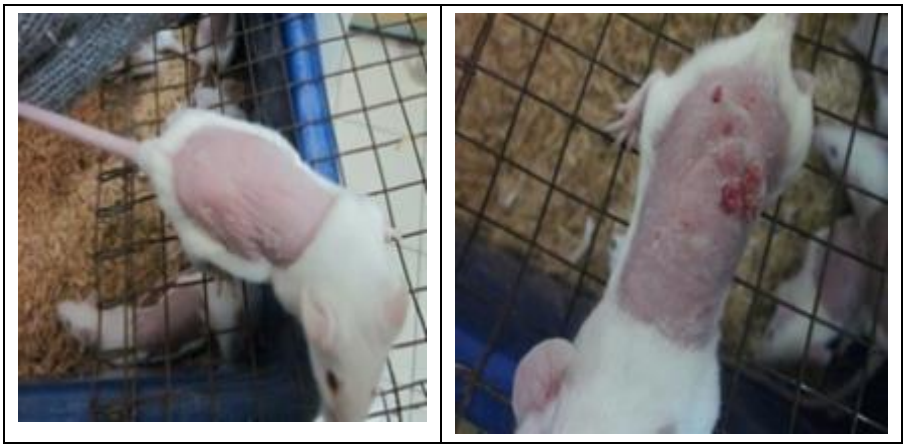
2.3 Analyzed data

The experimental result is indicated by the mean ± standard deviation. Measured variables were compared by analysis of variance one way. P-values < 0,05 (2-sided test) were considered statistically significant.

3. Results and discussion

3.1 Morphology of tumors

Tumor appearance in mice are shown in Fig. 1. Along with the full tumors, many papillomas are also visible.



A.Mice served as normal controls; B.Mice received topical application of DMBA 0,2% in acetone (200µl) followed by UVB UV B 0,4 J/cm^2 5x weekly for 8 weeks

Figure 1 Mice have shown without tumors (A) and with tumors (B)

3.2 Histopathological studies

The histological changes in the mice tumors and skin were studied with hematoxylin and eosin staining of the paraffin sections in treatment groups. Normal skin in the control group I showed normal histology. The uniformly arranged epidermal and dermal layers with normal layers of keratin over the epidermis were observed. In histopathological sections of DMBA-treated mice tumors (Fig 2). In skin tumors, there was dyskeratosis of the epidermis and abnormally thickened epidermis, with deposition of keratinocyte pearls observed in the dermis and epidermis [13].

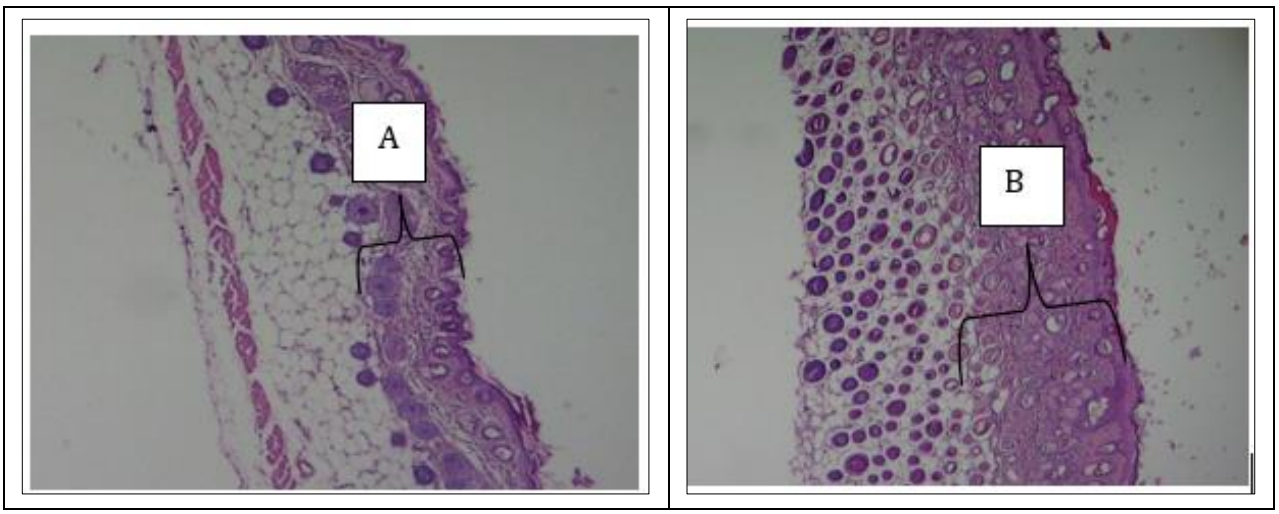
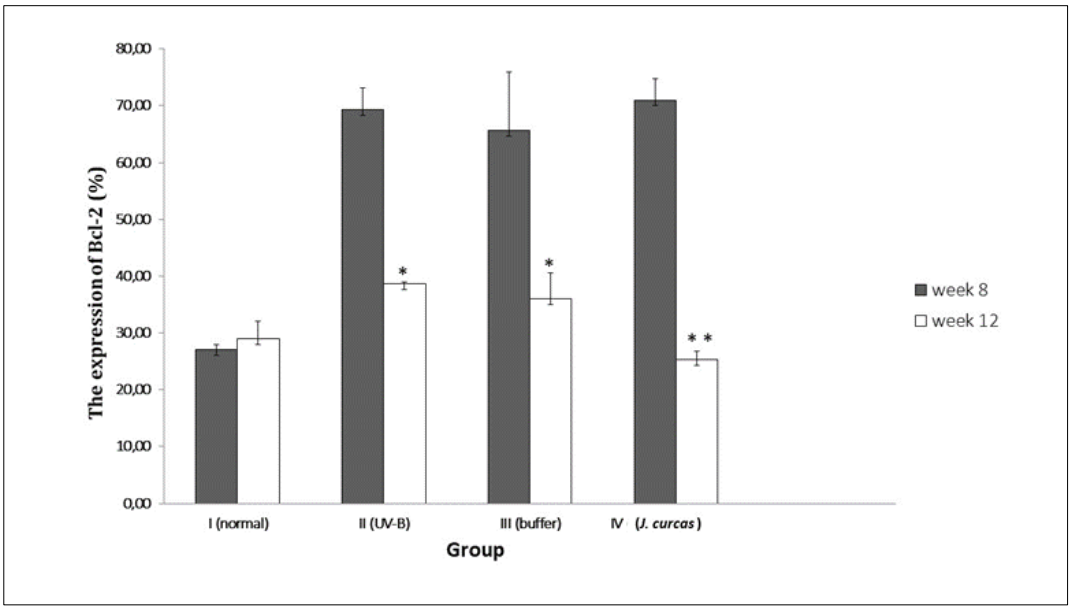


Figure 2 The histology of epidermis in the mice normal skin (A) and tumor skin (B) were studied with hematoxylin-eosin

The expression of Bcl-2 in skin tumors was indicated by brown in the cytoplasm membrane. The highest expression of Bcl-2 was found in the group with induced DMBA (38,67%). The expression of Bcl-2 in normal skin was 29%. Expression of Bcl-2 was found to be lowest in the group with treatment by protein fraction dose of 2 mg/0,1 ml (25,3 %).



Each result is represented as the mean ±SD. Significant differences compared with the control are represented as *P< 0.05; **P<0.01; ***P<0.001.

Figure 3 The expression of Bcl-2 in skin before(week 8) and after treatment (week 12) with *J.curcas*

There was a decrease in the expression of Bcl-2 in groups II, III, and IV. After being treated with *J.curcas*, the expression of Bcl-2 decreased from 71% to 25,3% (Fig.3). Compared with the other groups, the decrease of the expression of Bcl-2 was higher in group IV (treated with *J.curcas*), the differences were statistically significant (Table 1).

Table 1 The percentage of decreasing of expression Bcl-2 protein in various groups after being exposed to the DMBA and UV and treated with *J.curcas*

Group	Treatment	Decreased expression of Bcl-2 (%)
II	DMBA+UV-B	42,22
III	Buffer+DMSO	45,18
IV	*J.curcas*	64,32

Every living organism on the surface of the earth is exposed to the ultraviolet (UV) fraction of the sunlight. UV radiation can damage DNA and thus mutagenize several genes involved in the development of the skin tumor [14]. Ultraviolet (UV) radiation is the carcinogenic factor in sunlight; damage to skin cells from repeated exposure can lead to the development of tumors. The induction of skin tumors is mainly caused by the accumulation of mutations caused by UV damage [15]. Expression of P53 was found to be highest in the group with treatment by protein fraction dose of 2 mg/0,1 ml [12]. *Jatropha curcas* leaves were reported to have antimetastasis and antiproliferative on B16F10 lung melanoma induced by C57BL [11]. The previous study reported that curcin had antitumor [9] [10]. *Jatropha curcas* extract has a cytotoxicity effect on the HepG2 cell line [16].

The Bcl-2 gene (also known as Bcl-2) encodes for a mitochondrial protein thought to prevent apoptosis of normal cells. The protein has been detected by immunohistochemical procedures. BCL-2 is a gene of anti-apoptosis, the mechanism is possibly related to the effect of Ca^{2+} entering the cell, thereby regulating the signal transduction in the cells. BCL-2 is all members of the Bcl-2 gene family. Deregulation of Bcl-2 family members is also chiefly involved in skin carcinogenesis and response to cancer therapy [17] [18].

The research on gastrointestinal tumors and kidney tumors has found that high expression of Bcl-2 of inhibitor of apoptosis, induced tumor growth accelerated, poor prognosis, and poor response to treatment. The loss of Bcl-2 activity

may be related to tumor progression [15]. Studies and clinical trials have indicated that the over-activation of Bcl-2-related anti-apoptotic effects is associated with an increased risk of cancer development, progression, and prognosis. It may also contribute to the resistance to chemotherapy and radiation of different types of cancer. Thus, by increasing apoptotic sensitivity or reversing drug resistance, focusing on Bcl-2 inactivation has shown some strong therapeutic benefits [18][19]. The repair mechanism is the regulation of a cell-induced tumor. Specific decreasing of expression of Bcl-2 protein occurs in cells that require regulation of programmed death called apoptosis [20]. In this study, we find that the expression of Bcl-2, in tissues of treated skin with J.curcas was decreased after exposure to DMBA and UVB. The results showed that the protein isolated from the leaves of *Jatropha curcas* has the potential to stimulate apoptosis through decreasing of expression of Bcl-2.

4. Conclusion

The results showed the expression of Bcl-2 was found to be lowest in the group with treatment by protein fraction dose of 2 mg/0,1 ml. The highest expression was found in the group with induced DMBA and UVB. Decreased expression of Bcl-2 is a sign of apoptosis, so the protein *Jatropha curcas* has antitumor through induction of apoptosis.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

Statement of ethical approval

The experiment procedures were approved by the Ethics Committee in Medicine and Health Sciences, Faculty of Medicine, Jenderal Soedirman University.

References

[1] Leiter U, Keim U, Garbe C. Epidemiology of skin cancer: update 2019. Sunlight, Vitamin D, and Skin Cancer. 2020:123-39, https://doi.org/10.1007/978-3-030-46227-7_6

[2] Pfeifer GP. Correction to: Mechanisms of UV-induced mutations and skin cancer. Genome Instability & Disease. 2021 Aug;2(4):262, https://doi.org/10.1007/s42764-020-00009-8

[3] Wu Y, Xing D, Liu L, Gao B. Regulation of Bax activation and apoptotic response to UV irradiation by p53 transcription-dependent and independent pathways. Tumor Lett. 2008. 271:231-9.

[4] Qian S, Wei Z, Yang W, Huang J, Yang Y, Wang J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. Frontiers in oncology. 2022 Oct 12;12:985363, https://10.3389/fonc.2022.985363

[5] Fridman SJ, Lowe SW. Control of apoptosis. Oncogene. 2003. 22: 9030-9040

[6] Lucianò AM, Pérez-Oliva AB, Mulero V, Del Bufalo D. Bcl-xL: A focus on melanoma pathobiology. International journal of molecular sciences. 2021 Mar 9;22(5):2777. https://doi.org/10.3390/ijms22052777

[7] Saxena S, Sharma R, Rajore S, Batra A, Isolation and identification of flavonoid "Vitexin" from *Jatropha curcas* L., Jour Pl Sci Res, 2005. 21:116-17.

[8] Abdelgadir HA, Van Staden J. Ethnobotany, ethnopharmacology and toxicity of *Jatropha curcas* L.(Euphorbiaceae): A review. South African Journal of Botany. 2013 Sep 1;88:204-18.

[9] Lin, J., Yan, F., Tang, L., Chen, F., 2003, Antitumor effects of curcin seeds of *Jatropha curcas*, Acta Pharm acol Sin 2003 Mar; 24 (3): 241 -246.

[10] Ahmed, A., Ibrahim, M., El-said, M., Elsadek, B. Anti-Cancer Activity of Curcin and Latex Isolated from Jatropha Plant (*Jatropha curcas* L.). Journal of Agricultural Chemistry and Biotechnology, 2020; 11(11): 339-344. doi: 10.21608/jacb.2020.128902

[11] Oskueian E, Abdullah N, Saad WZ, Omar AR, Ahmad S, Kuan WB, Zolkifli NA, Hendra R, Wan Ho Y. Antioxidant, Antiinflammatory and Antitumor Activities of Methanolic Extracts from *Jatropha curcas* Linn, J. Med Plants Res., 2011. 5(1), 49-57.

[12] Baroroh, H., N., Warsinah, Harwoko, Activity of Protein Fraction from Jatropa curcas As Anticancer on p53 Expression in Mice Skin Exposed to DMBA and UVB. Jurnal Ilmu Kefarmasian Indonesia, Sept 2012, 10 (2), 138 - 143.

[13] Schwarz and Schwarz. Imunosuppretions Sistemik of Stratum corneum, J. lab. Clin. Med. 2006. 88: 577-579.

[14] Mao, P., Wyrick, J. J., Roberts, S. A., & Smerdon, M. J. UV-Induced DNA Damage and Mutagenesis in Chromatin. Photochemistry and Photobiology, 2017. 93(1), 216–228. https://doi.org/10.1111/php.12646.

[15] Mueller SA, Gauthier ME, Ashford B, Gupta R, Gayevskiy V, Ch'ng S, Palme CE, Shannon K, Clark JR, Ranson M, Cowley MJ. Mutational patterns in metastatic cutaneous squamous cell carcinoma. Journal of Investigative Dermatology. 2019 Jul 1;139(7):1449-58.

[16] Saleh ZM, Abdel Azeiz AZ, Mehany A, El-Swaify ZA. Anticancer and antimicrobial activity of Jatropha's leaves extracts. Egyptian Journal of Botany. 2023 May 1;63(2):621-34.

[17] Nys K, Agostinis P. Bcl-2 family members: essential players in skin cancer. Cancer letters. 2012 Jul 1;320(1):1-3.

[18] Qian S, Wei Z, Yang W, Huang J, Yang Y, Wang J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. Frontiers in oncology. 2022 Oct 12;12:985363.

[19] Linlin Zhang, Zaiming Lu, Xiangxuan Zhao, Targeting Bcl-2 for cancer therapy, Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, Volume 1876, Issue 1, 2021, 188569, ISSN 0304-419X, https://doi.org/10.1016/j.bbcan.2021.188569

[20] Luke JJ, Flaherty KT, Ribas A, Long GV. Targeted agents and immunotherapies: optimizing outcomes in melanoma. Nature reviews Clinical oncology. 2017 Aug;14(8):463-82. https://doi.org/10.1038/nrclinonc.2017.43