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by Indranila Kustarini Samsuria

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The Correlation between the Intrinsic and Extrinsic Molecular Markers in the Inhibition of the Lungs Carcinogenesis Growth by Mahkota Dewa Polyphenols on Balb/c Mouse

Theopilus Watuguly[1*], Kustarini Samsuria Indranila[2], Suharyo Hadisaputro[3], Edi Dharmana[4], Lyle E. Craker[5]

[1]Biologi Program, Department of Mathematic & Natural Science, University of Pattimura, Ambon, Indonesia
[2]Department of Clinical Pathology, University of Diponegoro, Semarang, Indonesia
[3]Departement of Internal Medicine, University of Diponegoro, Semarang, Indonesia
[4]Departement of Imunology dan Parasitology, University of Diponegoro, Semarang, Indonesia
[5]Department of Plant, Soil & Insect Sciences, University of Massachusetts, Amherst, USA
Email: *twatuguly@gmail.com

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Abstract

Phytochemical analysis shows that mahkota dewa fruit contains polyphenols. Mahkota dewa polyphenols have the ability to disrupt or reverse the stages of cancer or a carcinogenesis process by affecting the intracellular signaling tissue molecules involved in an initiation and/or promotion. This chemopreventive potential is highly anticipating. The use, effectiveness, and benefits of the polyphenol have been empirically and pre-clinically proved without side effects. A multivariate analysis using Spearman's test was conducted to examine the correlation between variables. Meanwhile, to determine which dependent variables played the strong role, a discriminant function analysis was performed with a significance level of $p < 0.05$ and a reliability level of 95%. The results showed that the mahkota dewa polyphenols acted as the suppressor agent by inducing apoptosis. This finding proved that polyphenols addition might result in death signal transduction through an apoptosis pathway which is either mediated by death receptor or mitochondria. The results of this study indicate that there is a relationship between apoptotic cell death mediated by death receptor and mitochondria through the administration of mahkota dewa polyphenols, it can be argued that mahkota dewa polyphenols can function in lung cancer chemoprevention in mice strain Balb/c.

Keywords

Polyphenols, Lungs Carcinogenesis, Molecular Markers, p53, Bcl-2, Bax,

Caspase-8, Caspase-9, Caspase-3

1. Introduction

The mechanism of apoptosis through the mitochondrial (intrinsic) pathway is initiated by the activation of tumor protein compressor 53, an important regulator mediated by mitochondria in response to cellular stress (Breuer *et al.* [1]). Protein 53 can increase proapoptotic protein (Bax, Bad, Bid, PUMA, Apaf-1) and/or decrease antiapoptotic protein (Bcl-2, Bcl-xL) (Hanahan and Weinberg [2]) in response to DNA damage (Vousden and Lu [3]) which spurred the release of cytochrome c from mitochondria (Fisher [4]). The cytochrome c release process will activate a number of caspases resulting in apoptosis (Sareen *et al.* [5], (Zimmermann *et al.* [6]).

While the mechanism of cell death through the death receptor pathway (extrinsic) is triggered through death activators that bind to the receptors on the cell surface concerned such as Tumor Necrosis Factor (TNF) and Ligand Fas (FasL) (Hanahan and Weinberg [2]). The Fas ligand pulls the Fas-associated death domain (FADD) in the intracellular direction which then captures procaspase-8 (Pop *et al.* [7], Viktorsson and Lewensohn [8]). Procaspase-8 transactivation will break down and active caspase-8 then activate procaspase-3 directly and activate active caspase-3 so that apoptosis occurs or enlarge the signal through splitting Bids (Garrido *et al.* [9]. According to Owen-Schaub, *et al.* [10], in addition to protein 53 it can activate the mitochondrial pathway, it can also activate the receptor death pathway so that it can activate caspase-8.

The process of apoptosis is important in eliminating cells that have damaged DNA that cannot be repaired and if the ability of apoptosis is decreased (Sreedhar and Csermely [11]) or the dysregulation of apoptosis results in cells surviving with DNA damage which in turn results in mutations 4 and is an important process in carcinogenesis (Crighton and Ryan [12], Rousselot and Garnero [13]). Apoptosis is an important event in the regulation of carcinogenesis and an appropriate balance between cell proliferation and cell death (apoptosis) determines the emergence of malignant neoplasia, hence both cellular processes are targets for chemopreventive intervention (Banerjee *et al.* [14]). For this reason, testing of medicinal plants as a chemopreventive agent capable of programmatically inducing cancer cell death by providing minimum side effects is indispensable in chemopreventive (Kintono and Pihie [15]).

Chemopreventive strategies aim at cancer intervention by administering natural ingredients that can inhibit, delay, and block or restore the process of carcinogenesis, which can be used to protect against carcinogenic exposure and reduce the risk of lung cancer (Banerjee *et al.* [16]). Many potential chemopreventive polyphenols can disrupt or reverse the carcinogenesis process by acting on intracellular signaling tissue molecules involved in initiation and/or promotion,

but phenolic compounds can also stop or reverse the stage of cancer development (Manson [17], Surh [18]). Its use, effectiveness and utilization have been proven without side effects both empirically and pre-clinical testing (Soeksmanto [19], Soeksmanto [20]).

Polyphenols are a very large general chemical class with >8000 known components (Finley [21]; Ross and Kasum [22]). Although the hidrophobic nature is found in most phenolic groups, glycoliation by sugars such as glucose, rhamnosa, galactose and arabinosa makes it soluble in water (Scalbert *et al.* [23]; Giovannelli *et al.* [24]). Based on phytochemical analysis, polyphenols are one of the compounds found in mahkota dewa plant (Lisdawati [25], Watuguly [26], Watuguly [27], Faried *et al.* [28], Sutiono *et al.* [29], Shahrzad and Bitsch [30]). *In vitro* test results conducted by Budijitno *et al.* [31] by looking at the anticancer effects of breast on C3H mice, were able to increase perforin expression and apoptotic index. Mahkota dewa polyphenols also appeared to have significant efficacy in increasing the apoptosis index.

Experimental studies conducted by Faried *et al.* [28] *in vitro* and in vivo on mahkota dewa extracts by looking at anticancer activity in human esophageal squamous cell carcinoma, are able to induce apoptosis in human esophageal cell tissue. In addition, the *in vitro* test of anticancer activity in C3H mice was able to increase apoptosis by using ethanol extracts mahkota dewa flesh 80 times the human dose induced by transplantation (Rahmawati *et al.* [32]). The results of other experiments carried out, showed that the mahkota dewa fruit of young and old had hypoglycemic activity with *in vitro* experiments with increased enzymes of alfaglucosidation and in vivo in mice (Sugiwati *et al.* [33]).

Mahkota dewa polyphenols are natural phenolics which are expected to have antioxidant properties and can potentially inhibit the growth of lung cancer cells. Studies by Frei and Higdon [34] show that polyphenols act as antioxidants and play a role in preventing cancer. As an antioxidant and anticancer, mahkota dewa polyphenols are expected to induce apoptosis in both the mitochondrial pathway and the death receptor pathway (Giovannini *et al.* [35]). The purpose of this study is to prove that mahkota dewa polyphenols can inhibit pulmonary carcinogenesis through molecular marker interactions in the intrinsic pathway and extrinsic pathway in Balb/c strain mice.

2. Materials and Methods

2.1. Study Area

The study began in February 2010-February 2011. The process of taking research material mahkota dewa plant (*Phaleria macrocarpa* [Scheff.] Boerl.) Was obtained from Ambon-Maluku, Indonesia. The process of plant determination is carried out at the Bogoriense Herbarium, Bogor, and West Java. Experimental animal research was carried out at the Integrated Research and Development Laboratory Unit IV, Experimental Animal Development Division of Gajah Mada University LPPT Unit IV-UGM, while extraction and fractionation were carried

out at LPPT Unit I. Fundamental considerations on the use of LPPT-UGM, because they had obtained KAN certification and ISO 9001-9002.

While the manufacture of paraffin blocks to immunohytochemistry and the reading of preparations were carried out at the Anatomical Pathology Laboratory of the Faculty of Medicine, Diponegoro University, Semarang, the Anatomical Pathology Laboratory, Yogyakarta Yogyakarta and the Anatomical Pathology Laboratory Dr. Sardjito Yogjakarta.

2.2. Design and Study Variables

This study is a pure in-laboratory experimental research with posttest control group design, using experimental animal mice Balb/c strains as research objects (Portney *et al.* [36], Gross and Mary [37], Campbell [38]). Independent Variable: in this study were polyphenols from the extract of the mahkota dewa fruit. Dependent Variables: in this study relating to apoptosis include: p53 expression, Bcl-2 and Bax expression, caspase-3, caspase-8 and caspase-9. Controlled Variables: strain, age, sex of test animals strain Balb/c. All animals are kept, handled and sacrificed according to the guidelines of the Health Research Ethics Commission (CEHR), Faculty of Medicine, Diponegoro University and Dr. Central Hospital Kariadi Semarang-Indonesia.

2.3. Research Samples

The number of samples used in the current research was determined based on the minimal number of samples used in a common experimental study which was $(t-1)(n-1) \geq 15$. The result of the calculation using the formula suggested five mice as the minimum number to be used in each treatment. One treatment group consisted of three terminated periods. Every terminated period employed 5 mice. This research, therefore, used 45 Balb/c mice in total. This figure had exceeded the criteria set by WHO for mutagenicity (5 animals).

2.4. IHC Staining

IHC painting uses antibodies labeled by the enzyme horseradish peroxidase. These antibodies will be bound to specific proteins. In cells that express proteins positively, the enzymes labeled in antibodies react with chromogen DAB to become a brown substrate, whereas cells with negative expressions will appear purple.

Immunohistochemical staining is scored according to the number of positively colored cells per 100 counted cells. Protein immunoreactivity 53, bax, Bcl-2, caspase-3, caspase-8 and caspase-9 are considered negative (0) when there is no staining; weak (1) when coloring is focal and rather intense; moderate/moderate (2) when about two-thirds of the cells are sufficiently colored; strong (3) when the majority of cells (>two thirds) are stained intensely. This inspection procedure is in accordance with that carried out by Mohan, *et al.* [39], Letchoumy *et al.* [40].

2.5. Statistical Analysis

Data on the expression of p53, protein Bax, protein Bcl-2, caspase-8, caspase-9 and caspase-3 proteins was collected in the form of ratio scales. Spearman's test was performed to test the correlation of the research variables. Meanwhile, to analyze which dependent variables played a strong role, a discriminant function analysis was conducted with a significance level of $p < 0.05$ and a reliability interval of 95%. Data collected statistically was analyzed using SPSS vers. 19.

3. Results

3.1. Histopathology of Lungs Carcinogenesis

Findings of this research confirmed that the administration of polyphenols extracted from the mahkota dewa plants could induce apoptosis during lungs carcinogenesis. Therefore, next step to be taken was to determine the effects of the polyphenols on genes expression related to apoptosis. Studies have suggested that pro and anti-apoptosis proteins play a significant role in an apoptotic process; thus, the current study aimed to investigate the effects of the mahkota dewa polyphenols on the expression of p53, pro-apoptosis Bax and anti-apoptosis Bcl-2, Caspase-3, -8 and -9 proteins in Balb/c mice. Observations were conducted thoroughly by calculating the number of cells that positively expressed p53, Bax, Caspase-8, Caspase-9 and Caspas3 proteins (brown colored) and the number of cells that expressed Bcl-2 (purplish blue) on the bronchial epithelium with MoAb anti-proteins staining.

The role of the mahkota dewa polyphenols in Bax protein expression was examined with an immunohistochemical analysis which had a purpose to evaluate a histopathological observation. The result of the observation on p53 protein expression suggested an increase in every group of the proteins. This finding showed that compared to the carcinogenic control group, the experimental group had more positive cells that could be found in the bronchial areas. An increase in the caspase-8, -9 and caspase-3 proteins expression was also reported by the experimental group However, the results of the observations on Bax and Bcl-2 proteins expressions in the experimental group indicated a decrease.

Observation using a microscope in 5 visual fields with a magnification of 400×. This observation starts from the left, right, upper, lower, and middle corners of the rat lung histology preparations. Each field of view was observed and a count was made of the number of cells. Results in pulmonary histopathology preparations on pulmonary features by H&E staining. The histopathology of lungs carcinogenesis was depicted in **Figure 1**.

There was an increase reported by expressions of p53, Bax, Bcl-2, caspase-8, caspase-9, caspase-3 proteins as well for both the control and experimental group on week 8, week, 17, and week 26. In the experimental group in particular, p53, Bax, caspase-8, caspase-9, and caspase-3 proteins expressions experienced an increase meanwhile Bcl-2 protein expression declined significantly (**Table 1**).

3.2. Multivariate Analysis Using Spearman's Correlation Test to Testing the Relationship between the Research Variables

Findings of the research suggested that the mahkota dewa polyphenols could inhibit lungs carcinogenesis on the experimental animals. This phenomenon could be a result of the dependency of the research variables tested in this research which were related to either cell proliferation or apoptosis. Therefore, Spearman's correlation test was conducted to examine the relationship between all dependent variables observed in the current research.

The result of the normality test indicated that all data distributed normally; thus, it was necessary to conduct data transformation analysis (log_10) of which results showed that data distribution was not normal. Spearman's correlation test was therefore performed and the result indicated the level (r = 0.00 - 0.199 (very weak); r = 0.20 - 0.399 (weak); r = 0.40 - 0.599 (medium); r = 0.60 - 0.799 (strong), and r = 0.80 - 1.000 (very strong) and direction of correlation between variables (positive (+) or negative (−) (see **Table 2**).

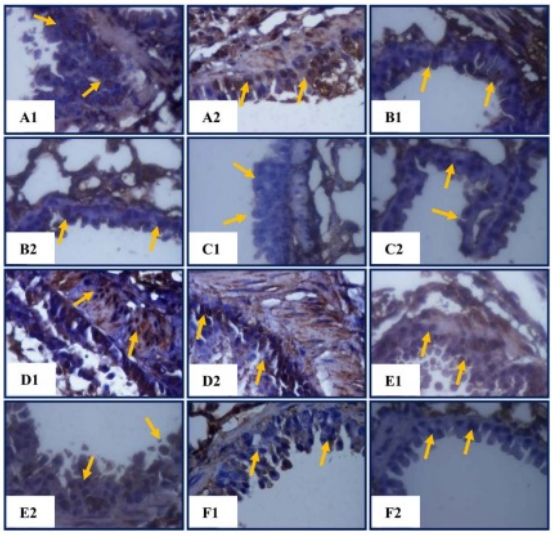

Figure 1. Histopathology in Balb/c Strain Mice. (A) The brown color of the cell nucleus shows the expression of protein 53 (Control = A1 and Treatment = A2). The nucleus of the cell appears brown oval. (B) The brown color of the cytoplasm shows Bax protein expression (Control = B1 and Treatment = B2). The nucleus of the cell looks round and oval brown (black arrow). (C) The brown color of the cytoplasm shows the expression of Bcl-2 protein (Control = C1 and Treatment = C2). The nucleus of the cell looks rounded oval blue-purple. (D) The brown color of the cytoplasm shows the expression of caspase-8 (Control = D1 and Treatment = D2). The nucleus of the cell looks round and oval brown (black arrow). (E) The brown color of the cytoplasm shows the expression of caspase-9 (Control = E1 and Treatment = E2). The core of the cell looks round oval blue-purple (black arrow). (F) Brown color in the cytoplasm shows the expression of caspase-3 (Control = F1 and Treatment = F2). The nucleus of the cell looks streaked with brown color. Observation of all cells with 400× magnification (histopathological pictures were taken based on permission from Watuguly TW, *et al.* [41] [42] [43] [44], Indranila KS, *et al.* [45].

Table 1. Description of the variable values changes in bronchial epithelium, apoptotic proteins of the control and experimental groups (mean ± SD).

No.	Group Variable C & T	N/n	Surgery Week			P
			8	17	26	
	P53					
1.	▪ Control	15/5	1.84 ± 0.29	2.32 ± 0.17	2.28 ± 0.33	0.000
	▪ Treatment		3.72 ± 0.22	5.20 ± 0.14	8.28 ± 0.33	
	Bax					
2.	▪ Control	15/5	4.04 ± 0.21	1.92 ± 0.17	1.92 ± 0.10	0.000
	▪ Treatment		5.96 ± 0.32	4.28 ± 0.30	4.68 ± 0.22	
	Bcl-2					
3.	▪ Control	15/5	5.80 ± 0.31	7.68 ± 0.43	9.64 ± 0.26	0.000
	▪ Treatment		5.12 ± 0.22	4.84 ± 0.08	7.36 ± 0.21	
	Caspase-8					
4.	▪ Control	15/5	0.32 ± 0.10	0.36 ± 0.08	0.72 ± 0.30	0.000
	▪ Treatment		0.64 ± 0.16	0.92 ± 0.22	2.04 ± 1.05	
	Caspase-9					
5.	▪ Control	15/5	0.32 ± 0.10	0.48 ± 0.22	0.28 ± 0.10	0.000
	▪ Treatment		0.56 ± 0.16	1.00 ± 0.20	2.60 ± 0.28	
	Caspase-3					
6.	▪ Control	15/5	0.28 ± 0.17	0.20 ± 0.14	0.56 ± 0.16	0.000
	▪ Treatment		0.60 ± 0.14	0.96 ± 0.16	2.52 ± 0.33	

Note: p value < 0.05. Kruskal Wallis Test.

Table 2. The results of the spearman's correlation test.

Correlations							
Variable	**P53**	**Bax**	**Bcl-2**	**Cas-8**	**Cas-9**	**Cas-3**	**IA**
P53	1	0.570**	−0.313	0.696**	0.798**	0.829**	0.876**
	.	0.001	0.092	0.000	0.000	0.000	0.000
Bax		1	−0.621**	0.379*	0.505**	0.687**	0.602**
		.	0.000	0.039	0.004	0.000	0.000
Bcl-2			1	−0.072	−0.348	−0.462*	−0.328
			.	0.705	0.060	0.010	0.077
Caspase-8				1	0.648**	0.715**	0.744**
				.	0.000	0.000	0.000
Caspase-9					1	0.864**	0.729**
					.	0.000	0.000
Caspase-3						1	0.830**
						.	0.000

The results of the correlation test on Bax, Bcl-2, Cas-8, -9, -3 proteins were (0.006; 0.000; 0.000 = significant); 0,191 (not significant); (0.017; 0.000 and 0.002 = significant). Each of the variables reported Spearman's correlation index as (r = −0.488; −0.624 = negative; medium and strong); (r = 0.754 = positive; strong); (r = −0.245; −0.433; −0.605; −0.533 = negative; weak, medium, strong and medium). The results of the correlation test on p53 and Bax, Bcl-2, Cas-8, -9, -3

proteins were (0.001 = significant); 0.092 (not significant); (0.000; 0.000; 0.000; 0.000 = significant). Each of the variables reported Spearman's correlation index consecutively as (r = 0.570 = positive; medium); (r = −0.313 = negative; weak); (r = 0.696; 0.798; 0.829; 0.876 = positive; strong, strong, very strong, and very strong).

The results of the correlation test on Bax and Bcl-2, Cas-8, -9, -3 (0.000; 0.039; 0.004; 0.000; 0.000 = Significant). Each of the variables reported Spearman's correlation index consecutively as (r = −0.621 = negative; strong); (r = 0.379; 0.505; 0.687 and 0.602 = positive; weak, medium, strong, and strong). The results of the correlation test on Bcl-2 and Cas-8, -9, -3 were (0.705; 0.060 = not significant); (0.010 = significant); (0.077 = not significant). Each of the variables reported Spearman's correlation index consecutively as (r = −0.072; −0.348; −0.462; −0.328 = negative; very weak, weak, medium, and weak).

The results of the correlation test on Cas-8 and -9, -3 proteins. Each of the variables reported Spearman's correlation index consecutively as (r = 0.648; 0.715 and 0.744 = positive; strong). The results of the correlation test on Cas-9 and Cas-3 proteins were significant (0.000). Each of the variables reported Spearman's correlation index consecutively as (r = 0.864 and 0.729 = positive; very strong and strong).

3.3. Discriminant Factors of the Research Variables

Multivariate and discriminant analysis could be performed on data which distributed normally. Otherwise, a normality test should be conducted. The initial analysis indicated 2 outlier variables; therefore, data transformation was necessary. The result of the data transformation analysis suggested that all variables were homogeneous and distributed normally. As a result, a discriminant function analysis could be conducted. This analysis was preceded by an equality test to ensure that all independent variables and covariance groups were equal.

The discriminant function analysis was conducted to investigate the interaction roles and contribution of dependent variables observed in both groups (control and experimental groups). The present research employed stepwise discriminant analysis where the variables were inserted one by one into the discriminant model. Meanwhile, the final analysis showed that six variables (p53, Bax, Bcl-2, caspase-8, and -3) could be processed for further analysis, the Fisher's linear discriminant functions analysis (see **Table 3**).

Table 3 and **Figure 2** presented values of each variable in the control and experimental groups which had been selected to proceed in the final analysis. To test, the accuracy of the discriminant functions classification, a cross-validated group test was conducted as shown in **Table 4**.

The results of the discriminant functions analysis indicated two cases, original group (5/5 × 100% = 100%) and cross validated group (5/5 × 100% = 100%). It can be concluded that both groups had the same functions. Output data in Table 5.14 presented that the accuracy of the model was 100.0% which suggested the

inhibition of the lungs carcinogenesis. To anticipate bias, leave-one-out-cross validation was performed and the result was 100.0%. It was then proved that the discriminant functions were very accurate. The original group variables included p53, Bax, Bcl-2, caspase-8, 9, and 3, meanwhile the cross validated group consisted of p53, Bax, Bcl-2, Cas-8 and -3. The results of the analysis, thus, indicated that caspase-9 could be excluded from the analysis because this variable had the most less intense interaction with p53, Bax, Bcl-2 and caspase-8 and -3 both in the control and experimental groups.

4. Discussion

The chemopreventive properties of the mahkota dewa polyphenols are assumed to be able to cause an apoptotic process indicated by cell's blebbing and shrinkage, increased permeability and decreased mitochondrial potential. An apoptotic cell will experience DNA damage which can be detected with TUNEL staining. According to Bonner *et al.* [46], the use of this method can result in high specificity to detect cells that undergo an apoptotic process.

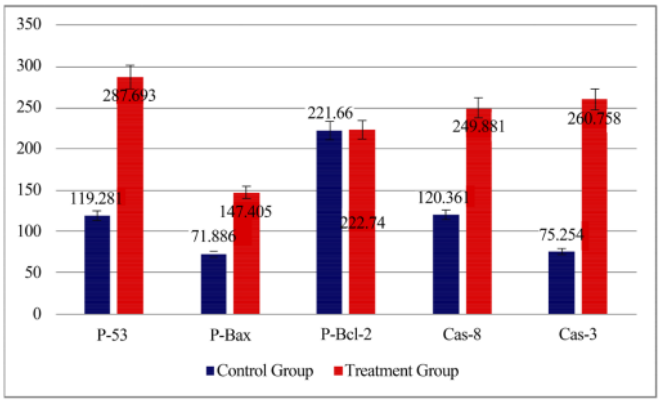

Figure 2. The contribution of the dependent variables in the control and experimental groups.

Table 3. Classification function coefficients. Fisher's Coefficients for p53, Bax, Bcl-2, Caspase-8 and Caspase-3.

Dependent Variable	Group	
	Control	Treatment
P-53	119.281	287.693
P-Bax	71.886	147.405
P-Bcl2	221.660	222.740
Cas-8	120.361	249.881
Cas-3	75.254	260.758
(Constant)	−1845.396	−3446.083

Note: Fisher's linear discriminant functions.

Table 4. The accuracy of the discriminant functions classification based on the classification results.

		Group	Predicted Group Membership		
			Control	Treatment	Total
Original	Count	Control	5	0	5
		Treatment	0	5	5
	%	Control	100.0	0.0	100.0
		Treatment	0.0	100.0	100.0
Cross-validated	Count	Treatment	5	0	5
		Treatment	0	5	5
	%	Control	100.0	0.0	100.0
		Treatment	0.0	100.0	100.0

It has been reported that EGCG can boost apoptotic index in human's epidermal carcinoma cells A431 (Kelly *et al.* [47]). According to Ahmad, *et al.* [48], apoptosis in human's colon adenocarcinoma HT-29 could be induced by EGCG. Apoptosis can also be triggered by theaflavin and in vivo EGCG. Either theaflavin or EGCG can inhibit cell proliferation and as a result improve apoptosis. This inhibition process significantly affects lungs carcinogenesis induced by BP in strain A mice (Banerjee *et al.* [14]). The current research suggest that an increase in the apoptotic index in the experimental group can be a result of the administration of the mahkota dewa polyphenols.

The results of the statistical analyses performed in this research indicated that polyphenols extracted from the mahkota dewa plants could inhibit lungs carcinogenesis in mice through cell proliferation and apoptotic mechanisms. Similarly, the correlation between Bax, Bcl-2, Cas-3 was also significant ($p = 0.000$) and strong ($r = 0.624$; 0.754; 0.605). These figures, therefore, the increase of Bax protein as well as the decrease of Bcl-2 which in turn activates caspase-3 in the apoptotic mechanism.

There was also a strong and significant relationship found between p53 and Bax, Cas-8, 9 and 3 proteins ($p = 0.001$; 0.000). Theories suggest that the activation of Bax protein by p53 will result in releasing c-cytochrome from caspase-9 and end up activating caspase-3 and caspase-8 on the death receptor. Mahkota dewa polyphenols extracted from the have been proved able to activate p53 protein so that caspase-8 is boosted and caspase-3 is triggered.

The correlation between Bax and Bcl-2 was significant ($p = 0.000$) and strong ($r = 0.000$). This finding suggest that the decreased Bcl-2 expression as an anti-apoptosis and the increased Bax expression as a pro-apoptosis lead to the release of c-cytochrome which initiates an assembly of Apaf-1 (Apoptotis protease-activating factor) and pro-caspase 9 to form an apoptosome. ATP is needed by Apaf-1 to recruit procaspase 9 through the so-called CARD (caspase recruiting domain). Then, procaspase-9 is autolitically broken down into active

caspase 9 which can activate pro-caspase 3 and disintegrate substrates and apoptosis. This finding indicate that apoptosis induction by the mahkota dewa polyphenols is not determined by the level of Bcl-2 and Bax expressions only, but by the ratio of both (Chao and Korsmeyer [49]).

The correlation between caspase-8, caspase-9 and caspase-3 was significant ($p = 0.000$) and strong ($r = 0.744$). These findings suggest that caspase-8, caspase-9 and caspase-3 play a significant role in inhibiting cell proliferation and inducing apoptosis. Therefore, the administration of the mahkota dewa polyphenols is effective to inhibit lungs carcinogenesis.

Based on the results of the discriminant function analysis, p53, Bax, Bcl-2, caspase-8 and -3 played a major role in mice lungs carcinogenesis. However, caspase-9 was reported to have no positive effect on the inhibition of the lungs carcinogenesis. Variable caspase-9 was known to establish weak interactions with other dependent variables despite the result of the non-parametric analysis (Kruskal-Wallis followed by Mann-Whitney test). Caspase-9 on mitochondrial pathways and Caspase-8 on death receptor pathways are considered as an initiator that is responsible to activate downstream, caspase-3, -6 and -7 effectors (Viktorsson K and Lewensohn [8]). Caspase-9 and Caspase-8 interact with each other to execute Caspase-3 so that an apoptotic process could happen. The interaction between variables of this research indicates that the role of Caspase-8 on the death receptor pathways is dominant in activating Caspase-3 and inducing apoptosis.

The results of the discriminant functions analysis also suggested that the apoptotic mechanism was initiated by cell proliferation indicated in the increase of p53 and Bax proteins and the decrease of Bcl-2 proteins that resulted in the permeability of mitochondria membrane. This process led to the release of c-cytochrome and the activation of Caspase-9, Caspase-8, and caspase-3 so that apoptosis can be induced. Therefore, it can be concluded that the administration of the mahkota dewa polyphenols is effective in improving the functions of the dependent variables (p53, Bax, Bcl-2, Caspase-8 dan -3).

Overall, the results of this research suggest that polyphenols extracted from the mahkota dewa plants could act as a suppressor agent by inducing apoptosis. In addition, the administration of the polyphenols can transduce death signal either through death receptor or mitochondrial pathways. Even though it has not been proved which pathway is dominant, it can be assumed that there is a crosstalk relationship between those two pathways at different levels. The interaction between the two pathways, therefore, can activate caspase-3 and induce apoptosis (Mohan *et al.* [39], Gupta [50]). In short, it can be said that the mahkota dewa polyphenols can perform an anti-cancer activity to inhibit lungs carcinogenesis the increase of p53, Bax, Bcl-2, caspase-8, caspase-9, and caspase-3.

Thus, it can be said that the mahkota dewa polyphenols have anti-cancer activity that can play a role in the process of inhibition of pulmonary carcinogenesis in experimental animals through the mechanism of inhibition of pulmonary

carcinogenesis, increased protein 53, Bax protein and Bcl-2 and increased caspase-8, caspase-9 and caspase-3.

5. Conclusion

The results of this study indicate that there is a relationship between apoptotic cell death mediated by death receptor (extrinsic) and mitochondria (intrinsic) through the administration of mahkota dewa polyphenols, it can be argued that mahkota dewa polyphenols can function in lung cancer chemoprevention in mice strain Balb/c.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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