THE EFFECT OF TURMERIC EXTRACT (CURCUMA LONGA) ADMINISTRATION IN KIDNEY HISTOPATHOLOGY FEATURES OF SPRAGUE DAWLEY RAT INDUCED BY 7,12-DIMETHYLBENZ(A)ANTHRACENE (DMBA)

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ABSTRACT

Objective: *The purpose of this study was to understand the effect of turmeric extract (Curcuma longa) on the histopathology features of male Sprague-Dawley rat's kidney induced with 7,12-Dimethylbenz(a)anthracene (DMBA).* ***Material & Methods:*** *This is an experimental design with post-test only control group design with 6 experimental groups and 30 samples. A comparison model was made by comparing the first group (K1) received no DMBA and the second group (K2) received DMBA alone. Four groups (K3, K4, K5, K6) received DMBA and different dosages of turmeric extract. The exposure was given for 14 days and histopathology features of the samples were assessed using the Gibson-Corley scoring system for Interstitial Tissue Damage. The data obtained was analyzed using One-sample Kolmogorov-Smirnov normality test and then analyzed with the Dunnett t-test.* ***Results:*** *The interstitial tissue damage in the negative control group (K1) and positive control group (K2/DMBA) was found to have a significant difference (p 0.000). There was no significant difference between DMBA only (K2) group and the group with turmeric administration groups (K3, K4, K5, K6) (P<0.05).* ***Conclusion:*** *DMBA can alter kidney histopathology features of male Sprague-Dawley rats. Administering turmeric (Curcuma longa) orally did not cause a change in kidney histopathology features of male Sprague-Dawley rats induced by DMBA.*

Keywords: *Curcuma longa, DMBA, histopathology features, Sprague-Dawley.*

ABSTRAK

Tujuan: *Tujuan dari studi ini untuk memahami efek dari ekstak kunyit (Curcuma longa) terhadap gambaran histopatologi pada ginjal tikus Sprague-Dawley laki – laki yang diinduksi oleh 7,12-Dimethylbenz(a)anthracene (DMBA).* ***Bahan & Cara:*** *Penelitian ini merupakan eksperimental dengan grup kontrol post-test dengan 6 grup eksperimen. Terdapat 30 sampel. Model perbandingan dibuat dengan membandingkan grup pertama (K1) yang tidak menerima DMBA dan grup kedua (K2) yang hanya menerima DMBA saja. Empat grup (K3, K4, K5, K6) menerima DMBA dan beberapa dosis ekstrak kunyit. Pemberian ekstrak dilakukan selama 14 hari dan gambaran histopatologis dari sampel dinilai menggunakan system skoring Gibson-Corley untuk kerusakan jaringan interstitial. Data yang didapat dianalisa menggunakan tes normalitas One-sample Kolmogorov-Smirnov dan Dunnett t-test.* ***Hasil:*** *Kerusakan jaringan interstitial pada grup kontrol (K1) dan grup kontrol positif (K2/DMBA) ditemukan perbedaan yang signifikan (p 0.000). Tidak didapatkan perbedaan yang signifikan pada grup DMBA saja (K2) dan grup dengan pemberian ekstrak kunyit (K3, K4, K5, K6) (P<0.05).* ***Simpulan:*** *DMBA dapat memepengaruhi histopatologi ginjal pada tikus Sprague-Dawley laki-laki. Pemberian kunyit (Curcuma longa) peroral tidak menyebabkan perubahan pada histopatologi ginjal tikus Sprague-Dawley laki-laki yang diinduksi dengan DMBA.*

Kata Kunci: *Curcuma longa, DMBA, gambaran histopatologi, Sprague-Dawley.*

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INTRODUCTION

Cancer is one of the leading causes of death in the world. In 2018, there was 18.1 millions newly diagnosed cancer with 9.6 millions death due to cancer where the incidence of renal cancer was 2.2% with mortality of 1.8% in 2018. Renal cancer is the 16th most common cause of cancer related death in the world. In Indonesia, the incidence of renal cancer is approximately 3/100.000 citizens with the comparison of male to female incidence of 3.2:1 and peak incidence at 60-70 years old. Until the date of this study, the most common identified renal malignancy was RCC (90%). The incidence of Renal Cancer Carcinoma (RCC) is about 3-5% from the incidence of all cancer.[1-3]

Renal cancer can be induced by chemical carcinogenic agent, for example 7,12-dimethylbenz(a)anthracene (DMBA). DMBA is a pollutant and a product obtained through pyrolysis of oil and biological materials, which was often found in cigarette smoke, vehicle emission, and incomplete combustion of coal and crude oil fuel. A kidney exposed to DMBA will show a change of cellular imaging form normal to abnormal. Corwin explained that high level of chemical substance and drugs may either renal structure or renal function.[4-5] For the past decades, the available therapy was focused on nephrectomy. Chemotherapy and immunotherapy often found toxic and not effective. The development of herbal treatment has becoming an alternative option in treating patients. Turmeric is one of the traditional herbs often used in Indonesia as an additive for food and herbal medicine. Turmeric contains active compound of curcuminoids, which composed of curcumin, dihydrocurcumin, demethoxycurcumin, triethyl curcumin, and bisdemethoxycurcumin, and acts as cyclooxygenase and arachidonic lipoxygenase catalyst and the metabolism of linoleate acid that hinders the development of cancer.[6-7]

Based on prior study conducted by Aggarwal (2014), curcumin inhibit lipid peroxidase induced by radiation and induce antioxidant enzyme which prevent the development of cancer cell.[8] Another study by Saksena (2018), showed that the use of turmeric extract may repair damaged kidney[7]. Duvoix et al (2013) explained that curcuminoid in turmeric extract have an anti-tumor, anti-inflammation, and antioxidant effect which decreases renal damage. Curcumin can be developed into a potent anti-cancer medicine.[9]

OBJECTIVE

The objective of this study is to see the effect of turmeric extract which was administered orally in different doses to a kidney that was damaged due to carcinogenesis material. The aim is to conclude whether oral administration of turmeric extract can affect the kidney changes in histopathology level hence can be considered as an alternative or adjuvant treatment to kidney cancer.

MATERIAL & METHODS

This was a true experimental study with post-test only control group design. This study was conducted in experimental laboratory of the Faculty of Medicine in Nusa Cendana University, and the process of obtaining histopathology slides was performed in the Anatomy Laboratory of Prof. Dr. W. Z. Johannes General Hospital in Kupang. the samples of this study were male Sprague Dawley rat which meet the inclusion criteria.

Turmeric extract was obtained through maceration and evaporation method. The turmeric was firstly washed, drained, and dried for 3 days until the turmeric was completely dried. The dried turmeric was then grounded into powder. The turmeric powder was then sifted and then soaked in 96% ethanol for 2x24 hours until it was homogenous. Maceration of turmeric powder was filtered using Whatman paper. The obtained filtrate is then evaporated using rotary vacuum evaporator to obtain a thick extract. This thick extract will be then boiled on a waterbath to eliminate ethanol. The final result of the extract will be stored at -4°C.

The samples of this study were 30 Sprague Dawley rats, which was divided into 6 groups. The first group is a comparison model (no induction of DMBA and turmeric administration). The second group was induced by DMBA 25 mg/kg BW dose for 7 days (positive control), and the kidney histopathology features was compared to the first group. Significant changes were observed between the first and the second group. The third group, until the sixth group was then induced by DMBA 25 mg/kg BW dose and turmeric extract, was administered with the dosage of 250 mg/kg BW, 500 mg/kg BW, 750 mg/kg BW, and 1000 mg/kg BW, respectively. Turmeric extract was administered orally for 14 days.

At the end of the study, the rat were terminated. The kidney tissue were obtained and

analyzed by a certified pathologist from the Department of Anatomical Pathology, University of Nusa Cendana. Each samples were observed for signs of renal histopathology changes and determined by Gibson-Corley score. The slides were examined by binocular Olympus CX21 microscope under 100x and 400x magnification.

The data was collected and analyzed. Data distribution was tested using One-sample Kolmogorov-Smirnov test (P>0.05). The first and second group was analyzed by t-test parametric test, and Dunnett t-test was performed to compare the 5 experimental groups.

RESULTS

The weight of the samples during the adaptation period was relatively constant, therefore no drop out in the study (Figure 1).

The kidney tissues were observed for specific histopathology changes referring to the Gibson-Corley score (Table 1), including the degree of hemorrhage, congestion, and infiltration of inflammatory cells, and the total score of each sample was documented (Table 2).

In order to know the DMBA induction resulted in kidney changes, the first group (K1) with no DMBA and no turmeric extract was compared to the second group (K2) that was induced by 25 mg/kg BW DMBA without any turmeric administration. The data was analyzed using t-test (Table 3).

Based on table 3 the interstitial tissue damage (either congestion, hemorrhage or infiltration of inflammatory cells) in the negative control group (K1) and positive control group (K2/DMBA) was found to have a significant difference. From this data, it was found that DMBA altered the histopathology feature of Sprague Dawley rats' kidney.

Table 1. Gibson-Corley Scoring System for Interstitial Tissue Damage.[11]

Variable	Score				
	0	1	2	3	4
Congestion	No congestion	Congestion ≤ 25%	Congestion 26-50%	Congestion 51 - 75%	Congestion ≥ 75%
Hemorrhage	No hemorrhage	Hemorrhage ≤ 25%	Hemorrhage 26-50%	Hemorrhage 51 - 75%	Hemorrhage ≥ 75%
Infiltration of inflammatory cells	No infiltration	Infiltration ≤ 25%	Infiltration 26-50%	Infiltration 51 - 75%	Infiltration ≥ 75%

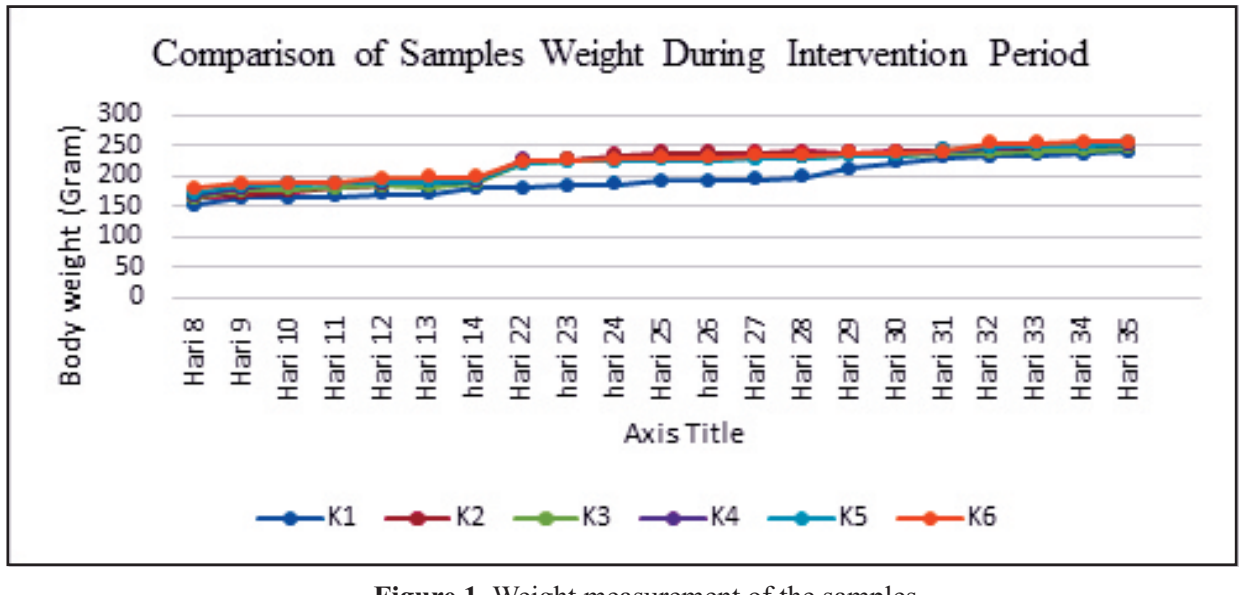

Figure 1. Weight measurement of the samples.

Comparison between the second group (K2/DMBA) and other experimental groups (K3, K4, K5, K6) were analyzed by Dunnett t-test (Table 4).

Table 4 showed that there was no significant difference between DMBA only (K2) group and the group with turmeric administration groups (K3, K4, K5, K6).

Table 2. Interstitial Tissue Damage Scoring.

Group	Hemorrhage	Congestion	Infiltration of Inflammatory Cells	Total
K1.1	0	1	0	1
K1.2	0	1	0	1
K1.3	0	1	0	1
K1.4	0	1	0	1
K1.5	0	1	0	1
K2.1	2	1	1	4
K2.2	1	2	1	4
K2.3	1	2	1	4
K2.4	1	2	0	3
K3.1	1	1	2	4
K3.2	1	1	1	3
K3.3	0	0	0	0
K3.4	0	0	0	0
K4.1	1	0	1	2
K4.2	0	0	3	3
K4.3	1	0	1	2
K4.4	2	2	3	7
K4.5	1	0	0	1
K5.1	1	0	1	2
K5.2	1	0	2	3
K5.3	1	0	3	4
K5.4	2	1	1	4
K5.5	1	0	1	2
K6.1	3	2	3	8
K6.2	1	0	1	2
K6.3	2	1	2	5
K6.4	1	1	3	5
K6.5	1	0	2	3

Table 3. The result of t-test analysis.

Comparison of K1 (Negative) and K2 (DMBA)	Asymp, sig	P	Distribution
	0.00	< 0.05	Normal

Table 4. Dunnett t-tests.

Group	Asymp,sig	P < 0.05	Distribution
K2-K3	.183	< 0.05	No significant Difference
K2-K4	.541	< 0.05	No significant Difference
K2-K5	.541	< 0.05	No significant Difference
K2-K6	.942	< 0.05	No significant Difference

DISCUSSION

In this study, the dose of the DMBA was 25 mg/kg BW was given for 7 days. The histopathology findings were congestion, hemorrhage, and infiltration of inflammatory cells. These findings were found due to the effect of DMBA which may cause damage to the DNA, accumulation of ROS, and mediate chronic inflammation. In the t-tests, the negative control group (no induction) and positive control group (DMBA induced) was found to have a significant difference, therefore exposure to DMBA may alter histopathology feature of rat's kidney which include hemorrhage, congestion, and infiltration of inflammatory cells.

Congestion is a passive process in which there was an accumulation of blood due to an abnormality of blood flow from the tissue. Systemic congestion happens when central blood flow was obstructed, such as in heart failure. Local congestion happens when external blood flow was obstructed by thrombus, embolus, infarct, ischemia, and tumor.[10] Congestion found in the group with DMBA was thought to be local congestion.

Hemorrhage is marked by an extravasation and blood accumulation in tissue and body cavity, and also leakage of blood from tissue. Hemorrhage was caused by chronic congestion and trauma due to obstruction of blood vessels. Hemorrhage was caused by the loss of blood vessels' integrity and external trauma.[11]

Inflammation is a body response to tissue damage due to toxic substance. Intratubular interstitial consist of several components such are dendritic cells, macrophages, lymphocytes, lymphocytic endothelial cells (inside the cortical intratubular interstitial) and fibroblast tissue.[12] Inflammatory cells found during interstitial damage were lymphocytes and macrophages. The most common inflammatory cells found in glomerulonephritis were lymphocyte and macrophage cells. Lymphocytes function as maintaining body immunity and macrophage plays an important role in inflammatory response.

It has been known that inducing DMBA initiate carcinogenesis in glomerulus, tubule, and interstitial tissue. Excessive amount of toxic substance in kidney will affect the morphology and function of the kidney. The effect could be necrosis, cell proliferation, infiltration of inflammatory cells, protein and other macromolecule leakages, atrophy, fibrosis, oedema, vacuolization, tubule, congestion, and hemorrhage. Histopathologic imaging of cancer found could vary, such as tumor necrosis, nuclear pleomorphism, multinucleate giant cells, and/or rhabdoid, and/or a very sarcomatous differentiation.[11] Prior study also stated that inducing DMBA to rat will create histological changes in glomerulus and tubules, such as atrophy, thickening of bowman capsule, protein accumulation, degeneration of cytoplasm, congestion, hemorrhage, and infiltration of inflammatory cells.[7]

In this study, the dose of turmeric used was 250 mg/kg (K3); 500 mg/kg (K4); 750 mg/kg (K5); 1000 mg/kg (K6). Statistical analysis using the Dunnett t-tests showed that turmeric extract did not have any effect to the histopathology features of the formerly DMBA induced kidney of the Sprague Dawley rats.

This result could be caused by several factors. The components of turmeric (antioxidant, anti-inflammation, and anticancer) have a low bioavailability in the plasma. This was due to rapid metabolism and slow absorption followed by rapid elimination and excretion which makes it hard to maintain the amount of turmeric in the plasma. Therefore, the effectivity of its component may decrease. Curcumin also has a low solubility and easily degraded which makes it harder to applied in clinical condition.[13]

Other possibility is due to several toxic substances in the curcumin that could have impaired the cells and tissues. Prior study by Wulandari and Kang showed that curcumin is dose-dependent and a group with high dose of turmeric extract could severe kidney damage.[14-15]

Another contributing factor is soil condition, which could affect the nutrition and the chemical components of turmeric. When selecting the turmeric in this study, it was from a highland region in East Nusa Tenggara province. According to the department of spices and herbs medicine, the chemical components of turmeric is higher in a lowland region.

CONCLUSION

The result of this study showed that inducing 7,2 dimethylbenz(a)antracene (DMBA) can alter kidney histopathology features of male Sprague-Dawley rats. Administering turmeric (Curcuma longa) orally did not cause a change in kidney histopathology features of male Sprague-Dawley rats induced by DMBA.

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