Gene p53 Mutations after the Induction of 7,12-Dimethylbenz(a)anthracene (DMBA) and Administration of Anti-Carcinogenesis Properties of Gynura procumbens in Sprague Dawley Rats

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Gene p53 Mutations after the Induction of 7,12-Dimethylbenz(a)anthracene (DMBA) and Administration of Anti-Carcinogenesis Properties of *Gynura procumbens* in Sprague Dawley Rats

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ABSTRACT

Objective: The aim of this research is to study *p53* gene mutations in the mammary gland of DMBA-induced Sprague Dawley rats treated with the ethanol extract of *Gynura procumbens*.

Methods: Sprague Dawley female rats aged 40 days were used. DMBA positive control animals were treated with 20 mg/kg bw DMBA; *Gynura*-extract positive control animals were treated with 300 or 750 mg/kgbw *G. procumbens* extract without DMBA treatment; experimental animals were treated with 300 or 750 mg/kg bw *G. procumbens* extract and 20 mg/kg bw DMBA, intragastric, while negative control animals neither treated with DMBA nor *G. procumbens* extract. Each group consists of 5 animals. Palpation and necropsy of mammary glands was conducted at the end of the experiment. Mutation of *p53* was studied in exon-6 of this gene, in triple or less, depended on available samples.

Result: *G. procumbens* extract generally suppress tumor incidence in DMBA treated animals. *p53* sequence showing some point mutations which also affect on its amino acid sequence, but it was not exclusively occurred in DMBA treated animals.

Conclusion: Based on this research we can not conclude that tumor incidence in this experiment was resulted by the mutation of *p53* gene.

1. Introduction

Cancer is the second cause of mortality in the world after cardiovascular diseases, while in Indonesia, cancer is the sixth leading cause of death. In industrialized countries breast cancer is the leading cause of death with an incidence of approximately 1-2% per year. Cancer administrations so far has not yet resulted in a good impact, from its complicated pathophysiological processes. The application of chemotherapy has not provided a permanent cure, as well. The usage of herbs as anti-cancer can be an alternative in cancer prevention and cure. In previous study, *Gynura procumbens* performed an anti-cancer potency. Five hundred mg/kg bw and 750 mg/kg bw ethanol extract of *G. procumbens* inhibited carcinogenesis in DMBA-induced rats [1]. The dose of 250 mg/kg bw and 750 mg/kg bw of *G. procumbens* ethanolic leaf extract given three times a week reduced the incidence of breast cancer by 10-20% in the DMBA-induced rat and decreased tumor multiplicity and cancer cell proliferation, as well [2].

It has been known that *G. procumbens* contains anti-tumor compounds which working through several possible mechanisms such as a signal transduction inhibitor process, stimulate cell cycle arrest, apoptosis, or inhibit metastasis [3]. Flavonoids, unsaturated sterols, triterpenes, polyphenols, essential oils are among compounds

contained in *G. procumbens*, L. Merr leaf [4,5] which may gives effect through two channels, i.e blocking agents and supressing agents. Blocking agents prevent carcinogens reaching the target either through inhibition of metabolic activity or inhibit the interaction with the target macromolecules such as DNA, RNA or proteins. Supressing agent inhibits the formation of malignant cells that have been initiated at the stage of promotion or progression [6].

p53 gene is a tumor suppressor gene. It interacts directly with a group of apoptosis regulators in Bcl-2 which triggers the activation of Caspase 3, 6 and 7, the executioners of caspase apoptosis [7]. This gene is also plays a role in genomic instability repair process. The failure of natural damage reparation will induce the increase of *p53* expression which in turn induces apoptosis [8]. TP53 polymorphisms (Arg72pro, rs1042552) known to play a role in apoptosis efficiency are associated with mutations in *p53* (Pro/Arg72 or Pro/Pro72 versus Arg/Arg 72; 2:25 OR, 95% CI 1:21 to 4:17) or G:C → T:A mutation in the TP53 (Pro/Arg72 or Pro/Pro 72 versus Arg/Arg 72; 2:42 oR, 95% CI 0.97-6.04) [9].

2. Materials and Methods

2.1 Animals

The experiment used females Sprague Dawley rat aged 40 days with body weight of 25 ± 60 g, divided into 6 groups. Each group placed in a cage and fed ad libitum.

DMBA positive control animals were treated with 20 mg/kg bw DMBA in corn oil; *Gynura*-extract positive control animals were treated with 300 or 750 mg/kg bw *G. procumbens* extract without DMBA treatment; experimental animals were treated with 300 or 750 mg/kg bw *G. procumbens* extract and 20 mg/kg bw DMBA, intragastric, while negative control animals neither treated with DMBA nor *G. procumbens* extract. Each group consists of 5 animals. The administration of *Gynura*-extract was done daily for 7 weeks from day-1; administration of DMBA was done twice a week for 5 weeks from 2nd week. Palpation was performed at week 8th for 11 weeks; and necropsy of mammary glands was conducted at week 19th. Mutation of *p53* was studied in exon-6 of this gene, in triple or less, depended on the available samples.

2.2 Gynura-extract preparation

G. procumbens leaves dried and crushed to form a powder. Five hundreds grams of leaf powder was macerated in 1.5L 96% ethanol for 72 hours, it then been concentrated in rotary evaporator to get a viscous extract. A total of 750 mg of ethanolic extract of *G. procumbens* were re-suspensed in 25ml of 0.5% CMC-Na, to obtain the 30 mg/ml extract concentration.

2.3 DNA extraction and p53 analysis

DNA extraction was carried out based on kit manufacturer protocol. Exon-6 of *p53* gene (*p53-e6*) was amplified by PCR in 94°C for one minute denaturation, 60°C for 2 minutes annealing, and 72°C for 2 min elongation for 30 cycles using forward and reverse primer 5'-CAACTGGCACACAGCTTCC-3' and 5'-GCCTCTGACTTATTCTTGC-3', respectively. PCR product was examined using electrophoresis in 1.5% agarose gel prior to sequencing procedure. Samples sequencing were done in Eijkman Institute for Molecular Biology in Jakarta. *p53-e6* sequence was analized using ClustalX software, amino acid sequence was analized using GeneDoc software.

3. Result

The incidence of tumors in DMBA-induced mice treated with *Gynura*-extract decreased by 20-40% compared to those without *Gynura*-extract treatment (Table 1). This result indicated the anti-cancer properties of *Gynura*-extract on DMBA-induced breast cancer. The amplification results of *p53-e6* is presented in Figure 1.

That result was supported with immunohistochemistry analysis of hepatocyte cells from female rats using CYP1A1 antibody. The cells showed a reducing expression of express CYP1A1 protein that treated with *Gynura procumbens*.

4. Discussion

The decrease of tumor incidence in *Gynura*-extract mice indicated the anti-cancer properties of *Gynura*-extract on breast cancer as reported before [1,2]. We have no capacity on explaining the result that the administration of 750 mg/kgbw *Gynura*-extract on DMBA-treated mice resulted in higher tumor incidence compared to those recieved 300 mg/kgbw *Gynura*-extract DMBA-treated mice. Further research need to be conduted to unveil this phenomenon.

The sequences of *p53* showing some gaps and undetected bases (N) in gynura 300mg/kg bw and DMBA positive controls (Suppl. 1). We suggest that the gaps were resulted by the different strain between animal we used and the reference animal

Table 1. Incidence of Mammary Gland Tumors in experimental Rats

Treatment	Incidence of Tumors (%)
Negative control	0
DMBA positive control	100
Gynura positive control (300 mg/kgbb)	0
Gynura positive control (750 mg/kgbb)	0
DMBA 20 mg/kgbb + Gynura 300 mg/kgbb	60
DMBA 20 mg/kgbb + Gynura 750 mg/kgbb	80

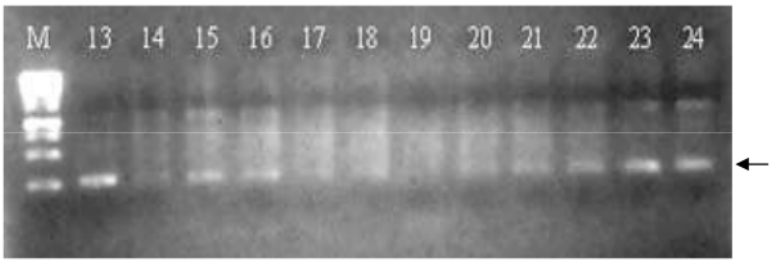

Figure 1. Amplification of *p53-e6*. Arrows indicates the target genes. M = Marker DNA, lanes 13-24 indicate the samples

(Gene Bank access code: gi[205947]). The "undetected" bases resulted on the changes of amino acid sequence (Suppl. 2). We suggest that these mutations led to the apoptosis of cancer cells, as reported by Mechanic *et al.* [9] and supported by the low incidence of tumors (60%) since p53 protein plays a role in inducing growth inhibition, DNA repair, or apoptosis in response to cellular stress [3].

Comparing to the differences in many bases comparing to the reference p53-e5-6, we suggest that there are more mutations occured throughout the samples sequences, both of controls and treatment groups. Added by the lost of some data due to the inavailability of the DNA extraction from the damaged tissue (we suggested that those tissues were in advanced stage of cancer development), these results need a further

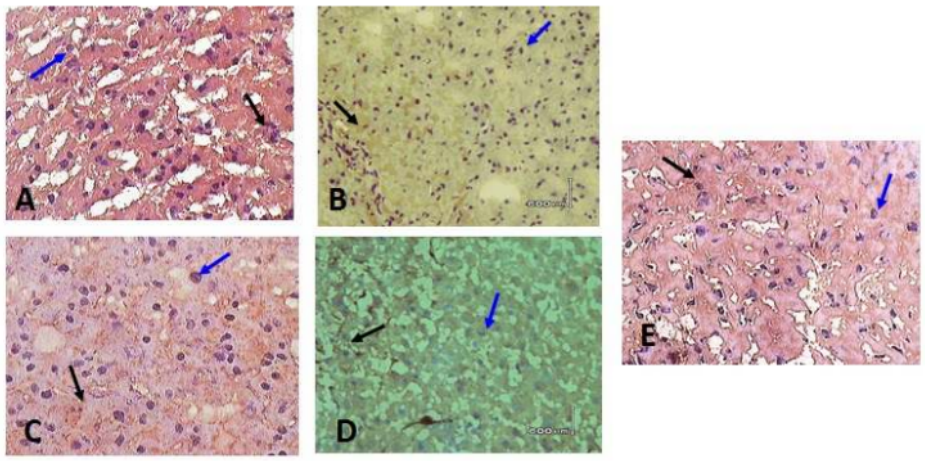

Figure 2. Immunohistochemistry of hepatocyte cells from female rats. (A) DMBA 20 mg/kg bb; (B) Extract 300 mg/kg bb+DMBA 20 mg/kg bb; (c) Extract 750 mg/kg bb+DMBA 20 mg/kg bb; (D) Corn oil solvent; (E) CMC Na solvent. Magnification 40x.

investigation on the role of *G. procumbens* in protecting and/or repairing the DNA mutation to support its efficacy as an anti-cancer medicinal plant,

5. Conclusion

Gynura procumbens extract lower tumor incidence of tumors in a dose of 300 mg/kg bw. Need a further investigation to unveil the exact role of *G. procumbens* protecting and/or repairing the DNA mutation with more available samples.

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Conflict of Interest

The authors report no conflicts of interest

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