RINGKASAN

Efek Fraksi *n*-Heksana : Etil asetat *Artemisia vulgaris* L.Terhadap Ekspresi Protein Ras, P53, PCNA C-myc dan Apoptosis Pada Sel Mukosa Rongga Mulut yang mengalami transformasi akibat induksi Benzopirene

Penelitian Eksperimental Laboratorik Pada Mencit (*Mus musculus*)

Karsinoma Sel Skuamosa Rongga Mulut merupakan jenis kanker yang paling sering ditemukan di rongga mulut. Faktor risiko utama terjadi keganasan di rongga mulut meliputi riwayat serta kebiasaan mengkonsumsi tembakau dan atau alkohol. Benzopirene adalah senyawa polisiklik aromatik hidrokarbon bersifat karsinogenik yang terdapat dalam asap rokok dan paling sering menyebabkan kanker di rongga mulut.

Tanaman Sudamala (*Artemisia vulgaris* L.) sering digunakan di masyarakat sebagai anti tumor pada organ pencernaan termasuk di rongga mulut, namun belum ada penelitian ilmiah dan belum ditemukan bahan aktif yang berperan sebagai anti kanker di rongga mulut. Banyak didapatkan spesies dari genus *Artemisia*, sedangkan yang banyak tumbuh di Indonesia adalah spesies *Artemisia vulgaris* L. Masalah dalam penelitian ini bahwa efek *Artemisia vulgaris* L. terhadap penurunan onkogenesis pada sel mukosa rongga mulut yang mengalami transformasi akibat induksi benzopirene belum jelas.

Penelitian ini bertujuan menjelaskan efek pemberian per oral fraksi *n*-heksana : etil asetat (3:7) *Artemisia vulgaris* L. terhadap penurunan onkogenesis pada sel mukosa rongga mulut mencit yang mengalami transformasi akibat induksi benzopirene.

Jenis penelitian yang dilakukan adalah penelitian eksperimental murni dengan menggunakan hewan coba mencit *(Mus musculus)* strain Swiss Webster (Balb/c) jantan, umur 2,5 bulan, berat badan berkisar 20-30 gram, terbagi menjadi lima kelompok masing masing 10 ekor mencit. Kelompok kontrol diberi pelarut benzopirene Oleum Olivarum selama 4 minggu lalu dilanjutkan pemberian pelarut fraksi CMC-Na 0,5 %. Kelompok 2 diberi benzopirene selama 4 minggu lalu dilanjutkan pemberian CMC-Na 0,5 %. Kelompok 3 diberi benzopirene selama 4 minggu lalu dilanjutkan pemberian fraksi 50 mg/kgbb. Kelompok 4 diberi benzopirene selama 4 minggu lalu dilanjutkan pemberian fraksi 100 mg/kgbb. Kelompok 5 diberi benzopirene selama 4 minggu lalu dilanjutkan pemberian fraksi 200 mg/kgbb. Pemberian benzopirene dilakukan 2 kali seminggu selama 4 minggu dengan paparan di rongga mulut mencit mukosa bukal sebelah kanan. Pemberian fraksi satu kali setiap hari selama 8 minggu secara per oral sesuai berat badan mencit. Setiap hari kondisi mencit dipantau, dilakukan penimbangan berat badan setiap minggu sekali dan pemeriksaan pertumbuhan tumor. Pada akhir minggu ke 12 mencit dimatikan dan diambil jaringan mukosa rongga mulut sebagai spesimen biopsi kemudian dilakukan pewarnaan TUNEL *assay* serta imunohistokimia pada Ras, P53 tipe normal, P53 mutan, PCNA dan C-myc. Data penelitian dianalisis dengan analisis multivariat (Manova). Bila terdapat perbedaan yang bermakna dilanjutkan uji Bonferroni dengan $\alpha = 0.05$ dan analisis diskriminan.

Pada data hasil perhitungan jumlah sel yang mengekspresikan protein P53 tipe normal, P53 mutan, Ras, PCNA, C-myc dan apoptosis dilakukan uji normalitas multivariat menunjukkan distribusi data dari seluruh kelompok adalah normal. Kemudian dilakukan uji homogenitas secara bersama sama dengan *Box's M test* dan secara individu dengan *Levene's test* yang menunjukkan tidak ada perbedaan

bermakna (p>0,05) sehingga memenuhi syarat uji asumsi untuk Manova. Terdapat perbedaan yang bermakna antar kelompok pada hasil analisis multivariat dan dari hasil uji Bonferroni terdapat perbedaan secara signifikan antara kelompok kontrol dan kelompok perlakuan (p< 0,05). Perbedaan secara signifikan juga didapat antar kelompok perlakuan dan meningkat sesuai peningkatan dosis. Didapat variabel sebagai pembeda (diskriminator) yang kuat pada seluruh kelompok adalah P53 mutan, P53 *wild*, PCNA dan apoptosis dengan kekuatan pembeda 100 %.

Kesimpulan penelitian ini adalah terdapat peningkatan apoptosis dan ekspresi P53 *wild* serta penurunan ekspresi P53 mutan, Ras, PCNA dan C-myc pada pemberian fraksi *n*-heksana : etil asetat (3:7) dari *Artemisia vulgaris* L. per oral terhadap sel mukosa rongga mulut mencit yang mengalami transformasi akibat induksi Benzopirene. Fraksi *n*-heksana : etil asetat (3:7) dari *Artemisia vulgaris* L. dapat mematikan sel yang mengalami transformasi tanpa merusak sel yang sehat.

SUMMARY

Effect of *n*-Hexana : Ethyl Acetate Fraction of *Artemisia vulgaris* L. on the Expression of Protein Ras, P53, PCNA, C-myc and Apoptosis in transformed oral mucosa cell caused induce Benzopirene

A Laboratory Experimental Study in Mice (*Mus musculus*)

Oral squamous cell carcinoma is the most frequent type of cancer found in oral cavity. The primary risk factor of malignancy in oral cavity includes the habit of consuming tobacco and or alcohol. Benzopirene is a carcinogenic polycyclic aromatic hydrocarbon compound found in cigarette smokes which most commonly causes cancer in oral cavity. The plant Sudamala (*Artemisia vulgaris* L.) is commonly used in the community as anti-tumor in digestive organ, including in oral cavity. However, there have been no scientific studies, while oral anti-carcinogenic active substances have not been found. The species are mostly from the genus Artemisia, while those generally grow in Indonesia is the species *Artemisia vulgaris* L. The problem studied in this research was that there was unclear effect of *Artemisia vulgaris* L. on the reduction of oncogenesis in transformed oral mucosa cells due to benzopirene induction. The objective of this study was to explain the effect of per oral administration of *n*-hexana : ethyl acetate (3:7) fraction of *Artemisia vulgaris* L. on the reduction of oncogenesis in transformed oral mucosa cells in mice due to Benzopirene induction.

This was an experimental study using male Swiss Webster (Balb C) strain mice (*Mus musculus*) as pure experimental animal. The mice were 2,5 months old,

with bodyweight of 20-30 grams. These animals were divided into five groups, each comprised 10 mice. Group 1, control, received benzopirene solvent Oleum Olivarum for 4 weeks, continued with 0.5 % CMC-Na fraction solvent. Group 2 received benzopirene for 4 weeks, followed with 0.5 % CMC-Na. Group 3 also received benzopirene for 4 weeks, followed with 50 mg/kgbw fraction. Group 4 received benzopirene for 4 weeks, followed with 100 mg/kgbw fraction. Group 5 received benzopirene for 4 weeks, followed with 200 mg/kgbw fraction. Benzopirene administration was done twice a week for 4 weeks with exposure in right buccal mucosa of mice oral cavity. The fraction was given once daily for 8 weeks per oral according to the mice's bodyweight. The condition was monitored every day, and birthweight scaling as well as tumor growth examination is done once a week. At the end of week 12 the mice were killed and oral mucosal tissue was taken for biopsy specimens. Tunnel assay staining and immunohistochemistry were undertaken for Ras, P53, PCNA and C-myc. Research data were analyzed with multivariant analysis (Manova). If significant difference was found, the test was continued with Bonferroni test with $\alpha = 0.05$ and discriminant analysis.

Data of cells expressing the protein of P53 wild type, P53 mutant, Ras, PCNA, C-myc and apoptosis, subjected to multivariate normality test indicating that data distribution of all groups was normal. Homogeneity test was simultanously with Box's M test and individually with Levene's test which showed no significant difference ($p > 0.05$) rendering it eligible for Manova. There was significant difference between groups in the result of multivariance analysis and the result of Bonferroni test showed significant difference between control and treatment groups ($p < 0.05$). Significant difference was also found among the treatment groups and increased along with the dose increment. The result of the discriminant analysis

ABSTRACT

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Ira Arundina

Sudamala (*Artemisia vulgaris* L.) is commonly used in the community as anti-tumor in digestive organ, including in oral cavity. However, there have been no scientific studies, while oral anti-carcinogenic active substances have not been found. This study was to explain the effect of per oral administration of *n*-hexana : ethyl acetate (3:7) fraction of *Artemisia vulgaris* L. on the reduction of oncogenesis in transformed oral mucosa cells in mice due to Benzopirene induction.

This was an experimental study using male Swiss Webster (Balb C) strain mice (*Mus musculus*) 2,5 months old, with bodyweight of 20-30 grams. Group 1, control, received benzopirene solvent Oleum Olivarum for 4 weeks, continued with 0.5 % CMC- Na fraction solvent. Group 2 received benzopirene for 4 weeks, followed with 0.5 % CMC-Na. Group 3 also received benzopirene for 4 weeks, followed with 50 mg/kgbw fraction. Group 4 received benzopirene for 4 weeks, followed with 100 mg/kgbw fraction. Group 5 received benzopirene for 4 weeks, followed with 200 mg/kgbw fraction. Benzopirene administration was done twice a week for 4 weeks with exposure in right buccal mucosa of mice oral cavity. The fraction was given once daily for 8 weeks per oral according to the mice's bodyweight. At the end of week 12 the mice were killed and oral mucosal tissue was taken for biopsy specimens. Tunnel assay staining and immunohistochemistry were undertaken for Ras, P53, PCNA and C-myc.

There was significant difference between groups in the result of multivariance analysis and the result of Bonferroni test showed significant difference between control and treatment groups. Significant difference was also found among the treatment groups and increased along with the dose increment. The result of the discriminant analysis showed that P53 mutant, P53 wild, PCNA and apoptosis as the discriminator variables with 100 % power test.

There is an increase of apoptosis and P53 wild type expression and the reduction of the expression of P53 mutant, Ras, PCNA and C-myc to squamous cells of mice oral cavity which transformed caused by benzopirene induced due to per oral administration of *n*-hexana : ethyl acetate (3:7) fractions from *Artemisia vulgaris* L. The fractions of *n*-hexana : ethyl acetate (3:7) is capable in killing cells which transformed without damaging the healthy ones.

Key words : *n*-hexana : ethyl acetate Fraction of *Artemisia vulgaris* L., Benzopirene, Oral Mucosa Cells