RINGKASAN

KAJIAN BIOLOGI MOLEKULER PADA KERUSAKAN SEL HATI SEBAGAI AKIBAT PROSES OKSIDATIF BIOTRANSFORMASI AFB_1

Yanwirasti

Aflatoksin B_1 merupakan hasil metabolit yang dihasilkan oleh Aspergillus Flavus, suatu kapang yang sering terdapat pada bahan makanan yang disimpan tidak menurut higiene kesehatan. Aflatoksin B_1 telah terbukti sebagai zat atau bahan hepatotoksik yang kuat, yang dapat menyebabkan kerusakan sel hati mulai dari yang ringan sampai kanker hati. Di dalam hati, Aflatoksin B_1 mengalami biotransformasi menjadi berbagai-bagai metabolit dengan katalisator sitokrom P-450. Sebagai efek samping biotransformasi AFB_1 oleh sitokrom P-450 ini akan dihasilkan produk-produk senyawa oksigen reaktif yang akan merusak sel hati melalui proses oksidatif. Dalam keadaan normal tanpa induksi pembentukan senyawa oksigen reaktif, pembentukan senyawa oksigen reaktif akan diredam oleh anti oksidan tubuh, tetapi bila induksi terus berlanjut, maka akan timbul suatu keadaan yang disebut stres oksidatif yang akan menimbulkan kerusakan oksidatif.

Di dalam tubuh senyawa oksigen reaktif akan menimbulkan kerusakan pada tiga jenis senyawa yang penting untuk mempertahankan integritas sel yaitu : lipid, protein dan DNA. Kerusakan oksidatif lipid akan menimbulkan peroksidasi lipid pada membran sel yang menyebabkan sel kehilangan integritas yang ditandai oleh meningkatnya peroksida lipid seperti MDA. Kerusakan oksidatif DNA akan menyebabkan teraktivasinya gen p53 yang akan mengaktifkan gen *down stream*, sehingga dapat menghambat pertumbuhan sel dan memicu terjadinya apoptosis. Bila induksi terus berlanjut, maka akan terjadi kerusakan dan perubahan sel yang ditandai dengan terjadinya displasia yang dapat berlanjut terus menjadi pertumbuhan sel kanker.

Walaupun para peneliti telah mendapatkan berbagai kerusakan sel hati akibat pemaparan Aflatoksin B_1, tetapi kajian mengenai perubahan biologi molekuler pada kerusakan sel hati sebagai akibat proses oksidatif biotransformasi AFB_1 menurut lama pemberian dan dosis yang berbeda dari Aflatoksin B_1 masih belum ada.

Penelitian ini bertujuan untuk mengungkap perubahan biologi molekuler kerusakan sel hati tikus putih akibat proses oksidatif pada biotransformasi AFB_1 menurut lama dan kadar pemberian.

Penelitian ini bersifat eksperimental murni dengan rancangan faktorial, karena mempergunakan 3 faktor lama pemberian dan 4 faktor dosis pemberian. Dalam penelitian ini digunakan 96 ekor tikus putih jantan (*Rattus Norvegicus Strain Wistar*) yang berumur ± 2 bulan dengan berat badan ± 180-200 g, yang dibagi atas 12 kelompok. Masing-masing kelompok terdiri atas 8 ekor tikus yang masing-masing kelompok diberikan Aflatoksin B_1 secara oral dengan dosis 0 µg,

10 µg, 15 µg dan 20 µg yang dilarutkan dengan 0,2 ml propilen glikol setiap hari selama 12 minggu, 16 minggu dan 20 minggu. Pada akhir percobaan, masing-masing tikus dikorbankan dan diperiksa kadar enzim superoksid dismutase jaringan hati dengan metode Wong (1989), enzim katalase jaringan hati tikus dengan metode Sinha (1972), malonaldehide jaringan hati dengan metode Uchiyama and Mihara (1978), kerusakan sel hati dengan sayatan histologi yang diwarnai dengan hematoksilin eosin, ekspresi protein p53 dengan pemeriksaan imunohistokimia, sel hati yang mengalami apoptosis dengan metode Tunnel Assay serta sel hati yang mengalami displasia atau pertumbuhan sel hati yang tak terkendali dengan sayatan histologi yang diwarnai dengan hematoksilin eosin. Hasil penelitian dianalisis dengan ANAVA dan kalau ada perbedaan dilanjutkan dengan Tukey HSD.

Analisis hasil penelitian ini menunjukkan bahwa : 1) ada perbedaan yang bermakna antara lama pemberian Aflatoksin B_1, 12 minggu dengan 20 minggu dengan dosis 10 µg dan 20 µg terhadap penurunan aktivitas enzim SOD dan enzim katalase jaringan hati, peningkatan kadar malonaldehid jaringan hati dan kerusakan sel hati serta displasia sel hati. 2) makin lama pemberian dan makin tinggi dosis Aflatoksin B_1 akan semakin menurunkan aktivitas enzim SOD jaringan hati dan enzim katalase jaringan hati, serta akan semakin meningkatkan kadar MDA jaringan hati, kerusakan sel hati, serta displasia sel hati, dimana displasia baru terjadi pada pemberian AFB_1 15 µg selama 16 minggu. 3) tidak ada perbedaan bermakna antara dosis 10 µg Aflatoksin B_1 dengan dosis 15 µg atau dosis 15 µg dengan dosis 20 µg dan lama pemberian Aflatoksin B_1 selama 12 minggu dengan 16 minggu atau 16 minggu dengan 20 minggu terhadap penurunan aktivitas enzim SOD dan katalase jaringan hati serta peningkatan kadar malonaldehid jaringan hati serta displasia sel hati. 4) tidak ada perbedaan bermakna antara lama pemberian dan dosis Aflatoksin B_1 terhadap sel hati yang mengalami apoptosis, bahkan tidak terdapat ekspresi protein p53 pada setiap lama pemberian dan dosis pemberian. 5) hasil interaksi dosis dan lama pemberian menunjukkan bahwa pemberian Aflatoksin B_1 selama 20 minggu dengan dosis 20 µg akan menyebabkan kerusakan sel hati yang sangat tinggi.

Dari hasil penelitian dapat disimpulkan bahwa Aflatoksin B_1 menimbulkan kerusakan sel hati melalui proses oksidatif yang terjadi akibat produk-produk senyawa oksigen reaktif yang dihasilkan biotransformasi Aflatoksin B_1 oleh sitokrom P-450. Semakin lama pemberian dan semakin tinggi dosis Aflatoksin B_1 yang diberikan akan semakin meningkatkan kerusakan sel hati yang meliputi degenerasi bengkak keruh, degenerasi lemak dan displasia serta menurunkan aktivitas enzim SOD dan katalase, serta meningkatkan kadar MDA.

SUMMARY

MOLECULAR BIOLOGY STUDY ON LIVER CELL DAMAGE DUE TO OXIDATIVE PROCESS OF AFLATOXIN B_1 BIOTRANSFORMATION

Yanwirasti

Aflatoxin B_1 (AFB_1) is a metabolic product of aspergillus flavus mushroom usually presents in food lacking hygienic precautions. The product has been proven to be a strong hepatotoxic substance, causing liver cell damage ranges from mild to liver cancer. In the liver, it undergoes biotransformation into different metabolites catalyzted by cytochrome, P-450. Side effect of P-450 catalyzation is the production of oxygen reactive species. In normal state, without the induction of reactive oxygen species production, the product would be overcome by body antioxidants. However, with the continuation of induction, oxidative stress resulted from increasing reactive oxygen species production would result in oxidative damages.

In the body, reactive oxygen species will damage three important components responsible for maintaining cell integrity, such as lipid, protein, and DNA. Oxidative damage on lipid results in lipid peroxidation on cell membrane, which in turn causes loss of cell integrity, confirmed by the presence of increased lipid peroxide such as malonaldehid (MDA). Oxidative damage on DNA causes activation of gene p53 which in turn activates downstream genes, resulting in the inhibition of cell growth and induction of apoptosis. If the induction continues, cell could be damaged and changed as indicated by dysplsia and followed by cancer.

Although the researchers have found different damages on liver cell due to exposure to Aflatoxin B_1, the study on biomolecular changes as the result of oxidative process on AFB_1 in term of length and doses of exposure have not been carried out.

This molecular biology study aims to disclose the damaging effect of oxidative process produced by different dosages and exposure times of Aflatoxin B_1 on white rat's liver cells. Using factorial design, in this experimental study, three exposure times and four doses of Aflatoxin B_1 were used. The experiment used 96 white rats (Rattus norvegicus) with age around eight weeks old and weight 180-200 grams, divided into four groups of 24 rats each, based on the dosages of Aflatoxin B_1 given. Each group was divided further into three subgroups of eight rats based on the length of exposure time to Aflatoxin B_1.

Four dosages of Aflatoxin B_1 were administered orally everyday into different groups, consisted of 0 µg, 10 µg, 15 µg, and 20 µg, dissolved in 0.2 ml propylene glycol. Three subgroups received the dosage for 12 weeks, 16 weeks, and 20 weeks. At the end of the experiment, the rats were sacrificed, and liver

enzymes were analyzed. Superoxide dismutase (SOD) was analyzed using Wong method, catalase using Sinha method, and malondialdehyde using Uchiyama and Mihara method. Liver cell damages were examined using histological slices stained by haematoxilin eosin. Expression of p53 protein was investigated using immunohistochemistry examination; liver cells with apoptosis were scrutinized using Tunnel Assay method. Cells with dysplasias or uncontrolled growth were examined using haematoxilin eosin stained slices. Data was analyzed using analysis of variance, and $P<0.05$ was considered to be significantly different.

There were significant differences between the effects of 12 weeks and 20 weeks exposure, and between dosage of 10 µg and 20 µg on reduction of enzymes SOD and catalase of liver tissue, increase in malondialdehyde of liver tissue, liver cell damage, and liver cell dysplasia. This also showed that increasing exposure time and dosages of Aflatoxin B_1 reduces SOD and catalase of liver tissue, and increases in malondialdehyde of liver tissue, liver cell damage and dysplasia. Moreover, dysplasia started on exposure to 15 µg AFB_1 for 16 weeks and it increases with increasing dosage and exposure time. There were no significant differences between 10 µg and 15 µg, between 15 µg and 20 µg dosages, between 12 weeks and 16 weeks, or between 16 and 20 weeks exposures, on reduction of enzymes SOD and catalase of liver tissue, increasing in malondialdehyde of liver tissue and dysplacia. No significant difference either was found between different exposure times or dosages on apoptosis of the liver cells. Moreover, no expression of p53 protein was found on any exposure time or dosages given. Analysis on the interaction between exposure time and dosage showed that 20 weeks exposure of 20 µg Aflatoxin B_1 would result in a very damaging effect on the liver cells.

The result of this study concluded that Aflatoxin B_1 damages liver cells by means of oxidative process due to reactive oxygen species generated by its biotransformation using cytochrom P-450. Increasing exposure time and dosages of Aflatoxin B_1 would increase the damage on liver cells such as cloudy swelling degeneration, fatty degeneration, necrosis, apoptosis and dysplasia.

ABSTRACT

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Keywords: Aflatoxin B_1, liver cell damage, oxidative process, AFB_1 biotranformation.