Efek proteksi kurkumin terhadap swelling, kegagalan potensial transmembran dan perubahan pola protein mitokondria hati tikus yang diinduksi oleh butilhidroperoksida tersier

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Abstrak

Kurkumin merupakan senyawa aktif utama dari berbagai Curcuma species. Sudah sejak lama rimpang berbagai Curcuma species seperti Curcuma Longa dan Curcuma xantharrhiza digunakan untuk pengobatan tradisional penyakit hati di Indonesia. Kemampuan kurkumin untuk bekerja sebagai hepatoprotektor telah diteliti secara in vitral dan in vivo. Kurkumin merupakan scavenger radikal bebas oksigen, seperti radikal anion superoksida, radikal hidroksil, dan radikal nitrogen dioksida. Aktifitas antioksidannya ditunjukkan dengan kemampuannya untuk menghambat peroksidasi lipid dalam homogenat otak tikus dan mikrosom hati tikus, serta mencegah deplesi kandungan -SH sel yang ditimbulkan akibat pemberian besi dan butilhidroperoksida tersier. Aktifitas antioksidan kurkumin tersebut dapat memegang peranan penting dalam kemampuannya sebagai hepatoprotektor. Untuk mengerti mekanisme proteksi kurkumin. perlu mengetahui pada tahap manakah proses yang menyebabkan kematian sel dapat dipengaruhi. Walaupun sempat diabaikan cukup lama, saat ini nampaknya mitokondria mempunyai peranan penting dalam kematian sel. baik dalam fisiologi maupun patologi. Akhir-akhir ini perhatian ditujukan pada kemungkinan peranan radikal bebas pada sejumlah penyakit termasuk penyakit hati. Oleh karena itu. penelitian ini dilakukan untuk meneliti efek kurkumin terhadap kerusakan oksidatif mitokondria hati tikus yang terisolasi yang diinduksi oleh butilhidroperoksida tarsier. suatu hidroperoksida organik yang sering digunakan untuk mempelajari stres oksidatif. Efek proteksi kurkurmin di nilai dari hambatannya terhadap swelling mitokondria. kegagalan potensial transmembran mitokondria dan perubahan pola protein mitokondria. Swelling mitokondria diikuti menggunakan spektrofotometer dengan mengukur penurunan absorbans pada 520 nm. Potensial transmembran mitokondria diamati menggunakan spektrofotometer pada dua panjang gelombang yang dinilai dari pergeseran panjang gelombang maksimum dan penurunan absorbans safranin O. yaitu dengan mengukur absorbans pada 516 nm dikurangi absorbans pada 495 nm. Pola protein mitokondria dinilai secara elektroforesis menggunakan SOS-PAGE. Hasil penelitian menunjukkan bahwa butilhidroperoksida tersier menyebabkan kerusakan mitokondria yang dinilai dengan adanya swelling mitokondria; kegagalan potensial transmembran mitokondria dan perubahan pola protein mitokondria yang diamati berupa pembentukan agregat protein dengan berat molekul tinggi dan berkurangnya jumlah kandungan protein pada pita 116 kD sebagai akibat ikatan silang thiol. Kurkumin 2500 µM hampir sempurna mencegah swelling mitokondria dan menghasilkan 85 % proteksi. sementara itu 79 % proteksi terhadap kegagalan potensial transmembran dicapai dengan penambahan kurkumin 250 uM. Kurkumin 3500 uM menghambat pembentukan agregat protein dengan berat molekul tinggi dan menghambat penurunan jumlah protein dengan berat molekul mendekati 116 kD. sebagai akibat adanya hambatan terhadap pembentukan ikatan silang thiol. Hal ini menunjukkan bahwa kurkumin memberikan proteksi terhadap kerusakan Kati oksidatif pada tahap organel mitokondria. ..... The Protective Effects of Curcumin on Swelling, Collapse of Transmembrane Potential, and Alterations of Protein Pattern of Rat Liver Mitochondria Induced by Tert-Butylhydroperoxide Curcumin is a major active compound of several Curcuma species. Many years ago the rhizomes of various Curcuma

species like Curcuma Longa and Curcuma xanthorrrizha are used for hepatic diseases in Indonesian traditional medicine. The potential of curcumin to act as hepatoprotector agent has been demonstrated in vitro and in vivo. Curcumin appears to be a potent scavenger of oxygen free radicals, such as super oxide anion radicals. hydroxyl radicals and nitrogen dioxide radicals. Its antioxidant activity has been shown by its capacity to inhibit lipid peroxidation in rat brain homogenates and rat liver microsomes, and by its ability to prevent the depletion of cellular -SH content by iron and tert-butylhydroperoxide. This antioxidant activity of curcumin may play an important role in its hepatoprotective ability. In order to understand the mechanism by which curcumin exerts its protective activity, it is important to know at which levels the process leading to cell death can be influenced. Although neglected for many years. it appears now that mitochondria have a major role on cell death, in both physiology and pathology. Current interest has focused on the possible role of free radicals in a wide range of diseases including hepatic diseases. Therefore, the present study was undertaken to investigate the effect of curcumin on oxidative damage of isolated rat liver mitochondria induced by tert-butylhydroperoxide. an organic hydroperoxide used frequently for studying oxidative stress. The protective effect of curcumin was assessed by studying its ability to inhibit mitochondrial swelling. collapse of mitochondrial Trans membrane potential and alterations of mitochondrial protein pattern. Mitochondrial swelling was followed using spectrophotometer by measuring the decrease of absorbance at 320 rim. Mitochondria] trans membrane potential was observed using a dual-wavelength spectrophotometer by measuring the absorbance of safranin 0 at 316 nm and at 495 nm showing a shift of the maximum wavelength of safranin O and the decrease of its absorbance. Mitochondrial protein pattern was assessed electrophoretically by using SDS-PAGE. The present study showed that tert-butylhydroperoxide caused mitochondrial damage as indicated by mitochondrial swelling, collapse of mitochondrial trans membrane potential. and alterations of protein pattern which were observed as the formation of high molecular mass protein aggregates and the decrease in protein content of 116 kD band due to thiol cross-linking. Curcumin at a concentration of 2500 uM almost completely prevented mitochondrial swelling providing 85 % protection. while 79 % protection against collapse of trans membrane potential was achieved with the addition of 250 uM curcumin. Curcumin at a concentration of 3500 uM inhibited the production of high molecular weight protein aggregates and the decrease of protein with molecular mass close to 116 kD due to inhibition of thiol cross-linking. This indicates that curcumin at concentrations of 250 uM - 3500 uM provided protection against oxidative liver damage at mitochondrial organelle level.