UNIVERSITI PUTRA MALAYSIA

HEPATOTOXIC EFFECT OF ANTIFUNGAL DRUGS ITRACONAZOLE AND FLUCONAZOLE ON RATS

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MASTER OF SCIENCE

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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

HEPATOTOXIC EFFECT OF ANTIFUNGAL DRUGS ITRACONAZOLE AND FLUCONAZOLE ON RATS

By

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January 2013

Chairman : Professor Muhammad Nazrul Hakim Abdullah, PhD

Faculty : Medicine and Health Sciences

Itraconazole and fluconazole are synthetic triazole antifungal from azole group. They exhibit fungistatic property by inhibiting ergosterol formation, an important structure in fungal membrane. These drugs have a broad spectrum antifungal activity and been used widely in treating *Candida albicans, Aspergillus spp., Cryptococcus neoformans* and many others. Unfortunately, these drugs were reported to cause liver toxicity in patients. The objective of this study is to study the hepatotoxicity effect of itraconazole and fluconazole in rats. *In vitro* toxicity test was done by using liver slice toxicity test method using normal rat's liver. Livers were harvested and sliced between 30-40 mg per slice. The liver slices were then incubated in 8 ml of complete RPMI-1640 media supplemented with itraconazole and fluconazole at different concentrations (0.0, 0.0001, 0.001, 0.01 and 0.1 mM). Incubation was done for 20, 40 and 60 minutes. After incubation was completed, liver slices were fixed in 10% formalin and prepared for hematoxylen and eosin (H&E) staining while incubation media was test for

aspartate transaminase (AST) and alanine transaminase (ALT) level. H&E staining evaluation of viable hepatocytes demonstrate that only incubation with itraconazole for 60 minutes, at all four concentrations gave significant low viable hepatocytes when compared to control group. While for AST and ALT level in incubation media, both itraconazole and fluconazole cause increment in time dependent pattern. Regarding the *in vitro* study, the difference between these two drugs was not significant. *In vivo* repeated dose treatment method was also conducted in this study. Rats were divided into one control and 6 treatment groups which treated with 10, 50 and 100mg/kg itraconazole or fluconazole. 1 ml treatment was given intraperitoneally, daily for 14 days. At day 15, the rats were sacrifice and liver were processed for mitochondrial permeability test (MPT), comet assay and immunohistochemistry staining. Result for MPT test suggests that itraconazole treatment lead to mitochondrial membrane pore formation in dose and time dependent pattern. Comet assay that been done to detect DNA damage showed that itraconazole caused more DNA damage compared to fluconazole especially at 50 and 100 mg/kg dosing. Immunohistochemistry staining show that bax protein was expressed especially at the higher dose for both drugs. In conclusion, itraconazole cause more hepatotoxicity effect compared to fluconazole.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KETOKSIKAN HATI YANG DISEBABKAN OLEH ANTI-KULAT ITRACONAZOLE DAN FLUCONAZOLE TERHADAP TIKUS

Oleh

AZHAR YAACOB

Januari 2013

Pengerusi : Professor Muhammad Nazrul hakim Abdullah, PhD

Fakulti : Perubatan dan Sains Kesihatan

Itraconazole dan fluconazole adalah anti-kulat triazole sintetik dari kumpulan azole. Mereka menghalang pertumbuhan kulat dengan menghalang pembentukan ergosterol, struktur yang penting dalam membrane kulat. Anti-kulat ini mempunyai spectrum aktiviti yang luas dan banyak digunakan untuk merawat jangkitan *Candida albicans, Aspergillus spp., Cryptococcus neoformans* dan lain-lain lagi. Malangnya, ubatan ini dilaporkan telah menyebabkan ketoksikan hati kepada pesakit. Objektif kajian kajian ini adalah untuk mengkaji kesan ketoksikan hati yang disebabkan oleh anti-kulat ini. Ujian ketoksikan *in vitro* telah dijalankan menggunakan kaedah potongan hati tikus yang normal. Hati diambil keluar dan dipotong antara 30-40mg setiap keping. Kepingan hati kemudiannya diinkubasi didalam 8 ml media RPMI-1640 yang ditambah dengan itraconazole dan fluconazole pada kepekatan yang berbeza (0.0, 0.0001, 0.001, 0,01 dan 0.1 mM). Inkubasi telah dilakukan pada tiga tempoh masa iaitu 20, 40 dan 60 minit.

Selepas inkubasi siap, kepingan hati diletakkan didalam 10% formalin dan bersedia untuk proses pewarnaan histologi *hematoxylin & eosin* (H&E) manakala media inkubasi diambil untuk ujian aras *aspartate transaminase* (AST) dan *alanine transaminase* (ALT). Penilaian hepatosit menunjukkan hanya inkubasi dengan itraconazole selama 60 minit memberikan keputusan yang signifikan dibandingkan dengan kumpulan kawalan untuk setiap kepekatan. Untuk ujian AST dan ALT, kedua-dua anti-kulat menyebabkan kenaikan paras AST dan ALT apabila masa inkubasi bertambah. Untuk kajian *in vitro*, perbezaan antara dua anti-kulat tersebut adalah tidak signifikan. Bagi ujian *in vivo*, kaedah *repeated dose treatment* juga dijalankan dalam kajian ini. Tikus telah dibahagikan kepada satu kumpulan kawalan dan 6 kumpulan yang dirawat dengan 10, 50 dan 100mg / kg itraconazole atau fluconazole. 1 ml rawatan diberikan secara suntikan ke ruang *intraperitoneal*, setiap hari selama 14 hari. Pada hari ke 15, tikus-tikus telah dimatikan dan hati telah diproses untuk ujian *mitochondrial permeability* (MPT), *comet assay* dan pewarnaan immunohistokimia. Keputusan ujian MPT menunjukkan bahawa rawatan itraconazole membawa kepada pembentukan liang pada membran mitikondria dalam corak bergantung kepada masa dan dos. Comet assay yang dijalankan untuk mengesan kerosakn DNA menunjukkan itraconazole menyebabkan lebih banyak kerosakan DNA berbanding fluconazole, terutamanya pada dos 50 dan 100mg/kg. Pewarnaan immunohistokimia menunjukkan protin bax dibebaskan didalam kedua-dua anti-kulat, terutamanya pada dos yang tinggi. Kesimpulannya, itraconazole menyebabkan ketoksikan hati yang lebih banyak berbanding fluconazole.

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I certify that an Examination Committee met on 22nd January 2013 to conduct the final examination of Azhar Yaacob on his Master of Science thesis entitle "Hepatotoxic Effect of Antifungal Drugs Itraconazole and Fluconazole in Rats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded with the relevant degree.

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DECLARATION

I hereby declare that this thesis is based on my original work except for quotations and citations, which have been dully acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

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AZHAR YAACOB

Date: 21 January 2013

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