UNIVERSITI PUTRA MALAYSIA

TOXICITY OF ANTIFUNGAL DRUGS ITRACONAZOLE AND FLUCONAZOLE IN RATS

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By

NOR SHAHIDA ABDUL RAHMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

"Dedicated especially to my parents Abdul Rahman Mat and Tuan Zaharah Tuan Yusoff, sisters, brothers and all those individuals behind the scenes who make me possible to complete my study successfully."

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Itraconazole and Fluconazole are the newer antifungal drugs that have been used for several years. Both these drugs have a broad-spectrum antifungal activity and currently are used to treat infections caused by *Candida albicans, Aspergillus spp., Paracoccidioides brasiliensis, Sporothrix schenckii, Histoplasma capsulatum, Cryptococcus neoformans* and many others. The objective of this study is to investigate the *in vitro* and *in vivo* cytotoxicity of these two antifungal drugs. The *in vitro* and *in vivo* cytotoxicity of fluconazole and itraconazole were studied in thirty eight male Sprague Dawley rats. Freshly isolated rat hepatocytes were obtained for the *in vitro* treatment of fluconazole and itraconazole using a liver perfusion technique. The cell viability test was done by trypan blue exclusion. As a result, both fluconazole and itraconazole cause a reduction in cell viability of hepatocytes. However, itraconazole exerted its cytotoxicity more than fluconazole in both time- and dose-dependent manner. Meanwhile, cytotoxicity of itraconazole was reduced significantly by Phenobarbital pretreatment. Phenobarbital did not have any effect on the

cytotoxicity induced by fluconazole. *In vivo* studies revealed that rat's liver and kidney treated with repeated-doses of itraconazole showed a significantly higher in total protein in liver and kidney and significant increase in serum ALP and ALT activity. This is in agreement with histological findings that the rat treated with repeated-doses of itraconazole showed severe histological features compared to fluconazole. Morphological changes such as inflammation and fibrosis of liver were frequently seen in repeated-doses of itraconazole. This present study suggests that Phenobarbital plays a role in the cytoprotection of hepatocytes to itraconazole-induced but not fluconazole-induced cytotoxicity *in vitro*.

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

KETOKSIKAN DADAH ANTI-KULAT ITRACONAZOLE DAN FLUCONAZOLE DALAM TIKUS

Oleh

NOR SHAHIDA ABDUL RAHMAN

Mac 2004

Pengerusi: Professor Madya Muhammad Nazrul Hakim Abdullah, Ph.D.

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Itraconazole dan fluconazole tergolong di antara dadah anti-kulat yang baru diperkenalkan dan telah digunakan sejak beberapa tahun yang lalu. Kedua-dua dadah anti-kulat ini telah digunakan secara meluas terutamanya dalam merawat jangkitan yang disebabkan oleh *Candida albicans, Aspergillus spp., Paracoccidioides brasiliensis, Sporothrix schenckii, Histoplasma capsulatum, Cryptococcus neoformans* dan sebagainya. Objektif kajian ini adalah untuk mengetahui ketoksikan kedua-dua dadah ini secara *in vitro* dan *in vivo*. Di dalam kajian ini tiga puluh lapan ekor tikus jantan dari spesies Sprague Dawley telah digunakan. Pengasingan sel hepatosit tikus segar dapat dibuat untuk digunakan dalam rawatan *in vitro* oleh fluconazole dan itraconazole dengan menggunakan teknik perfusi hati. Ujian untuk menguji sel-sel hidup dibuat menggunakan tripan biru. Fluconazole dan itraconazole menyebabkan penurunan kepada bilangan sel hepatosit yang hidup. Walaubagaimanapun, itraconazole menunjukkan kesan ketoksikan yang lebih tinggi berbanding fluconazole dalam keadaan berkadar langsung dengan masa dan dos.

Sementara itu, ketoksikan yang disebabkan oleh dadah anti-kulat itraconazole dapat dikurangkan dengan menggunakan Phenobarbital. Phenobarbital tidak mempengaruhi ketoksikan yang disebabkan oleh fluconazole. Kajian *in vivo* juga menunjukkan terdapat peningkatan dalam jumlah protein dan aktiviti serum ALP dan ALT di dalam hati dan buah pinggang tikus yang diberi suntikan itraconazole secara berulang-kali. Secara histologinya, tikus yang diberi suntikan itraconazole secara berulang-kali menunjukkan kesan ketoksikan yang ketara terhadap hati dan buah pinggang tikus jika dibandingkan dengan tikus yang diberi suntikan fluconazole secara berulang-kali. Perubahan morfologi seperti keradangan dan fibrosis pada hati telah dilihat dengan kerap dalam suntikan itraconazole secara berulang-kali. Dengan itu, kajian ini telah menunjukkan bahawa phenobarbital memainkan peranan dalam mengurangkan ketoksikan yang dihasilkan oleh itraconazole tetapi tidak mempengaruhi ketoksikan yang disebabkan oleh fluconazole secara *in vitro*.

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