
**UNIVERSITI PUTRA MALAYSIA**

# CYTOTOXIC EFFECT OF GIRINIMBINE ON HEPG2 CELLS

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# CYTOTOXIC EFFECT OF GIRINIMBINE ON HEPG2 CELLS

**By**

**SUVITHA SYAM MOHAN**

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

**September 2011**

# DEDICATION

*THIS THESIS IS DEDICATED TO*

*MY BELOVED HUSBAND SYAM MOHAN*
*MY LOVELY SON ADITHYA MOHAN*
*PARENTS AND PARENTS IN LAW*
*ALL MY TEACHERS AND LECTURERS*
*ALL MY SOULMATES AND KINDHEARTED FRIENDS*
*AND*
*TO EVERYONE WHO BELIEVED IN MY ABILITIES AND ALWAYS INSPIRED ME IN MAKING SOME OF MY GOALS COME TRUE*

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**CYTOTOXIC EFFECT OF GIRINIMBINE ON HEPG2 CELLS**

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**Chairman: Ahmad Bustamam Abdul, PhD**

**Faculty: Institute of Bioscience**

Girinimbine, a naturally occurring carbazole alkaloid, has been shown to possess wide range of pharmacological effects. However, to date, there is no literature evidencing the anticancer effect of this compound in human hepatocellular carcinoma (HCC). Here, we report that *in vitro* treatment of HepG2 cells (HCC cell line) with girinimbine inhibited cell proliferation and induced cell death in a dose-dependent and time-dependent manner which were analyzed by MTT and LDH assay ($IC_{50}$ 61 ± 2.3 µM, 56 ± 3.6 µM, and 40 ± 2.7 µM for 24, 48 and 72 h respectively). Girinimbine induced HepG2 cell death was identified by morphological features of apoptosis with the aid of Hoechst 33342 dye. The DNA analysis of girinimbine treated HepG2 cells with agarose electrophoresis resulted in significant DNA fragmentation with an increase in time dependent manner. There was 0.4 units (OD) ($p<0.05$) time-dependent increase in caspase-3 activity. Further, girinimbine also

induced accumulation of cells in $G_0/G_1$ phase (approximately 7.5 % ($p<0.05$) compared to control cells) in the HepG2 cell cycle progression. The intracellular level of reactive oxygen species (ROS) in HepG2 cells increased time-dependently after girinimbine treatment. The initial level of ROS in HepG2 cells was 105.20 ± 5.26 % which reached 132.70 ± 6.63 % ($p<0.05$) after 3 h. The intracellular antioxidant, GSH level after an initial elevation to 30 % to that of control decreased to 20 % after girinimbine treatment at 3 h. Mitochondrial damage, also increased from by girinimbine treatment as observed by the loss of mitochondrial membrane potential in flow cytometric analysis. The loss of mitochondrial membrane potential for control cells was 4.30 ± 0.21 %, while after girinimbine treatment the mitochondrial membrane potential became 25.30 ± 1.26 % at 3 h. The comet assay revealed a significant ($P<0.05$) 5-fold increase than the control upon exposure to 100 μM girinimbine after 3 h incubation. Girinimbine also induces Hsp 70 and Hsp 90 expression in a dose-dependent manner up to concentration of 100 μM and time -dependent manner after 24 h incubation. However, pretreatment of antioxidants ascorbic acid and catalase showed decrease in ROS level, Hsp level and mitochondrial damage, oxidative DNA damage, but an increase in GSH level. All these events are happening at an early stage (i.e., at 3 h after girinimbine treatment itself), suggesting the oxidative stress mechanism in inducing apoptosis in HepG2 cells. Taken together, these results strongly support the hypothesis that, after exposure, girinimbine suppressed the growth of HepG2 cells via induction of $G_0/G_1$ phase arrest and oxidative stress mediated apoptosis driven by mitochondrial pathway.

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

**KESAN SITOTOKSIK GIRINIMBINE PADA SEL-SEL HEPG2**

Oleh

**SUVITHA SYAM MOHAN**

**September 2011**

**Pengerusi: Ahmad Bustamam Abdul, PhD**

**Fakulti: Institut Biosains**

Girinimbine adalah carbazole alkaloid semulajadi, telah terbukti memiliki pelbagai kesan farmakologi. Walau bagaimanapun, sehingga kini, tidak ada kajian membuktikan kesan antikanser bahan ini dalam hepatocellular carcinoma (HCC) manusia. Rawatan girinimbine in-vitro, ke atas HepG2 sel-sel (HCC sel-sel) telah menghalang proliferasi sel dan merangsang kematian sel mengikut dos dan masa dengan menggunakan analisis kaedah MTT dan LDH ($IC_{50}$ 61 ± 2.3 μM, 56 ± 3.6 μM, dan 40 ± 2.7 μM untuk 24 jam, 48 dan 72 masing-masing). Girinimbine merangsang kematian sel HepG2 dikenalpasti melalui ciri-ciri morfologi apoptosis dengan bantuan pewarna Hoechst 33342. Analisis DNA HepG2 sel yang dirawat dengan girinimbine dengan elektroforesis agarose menunjukkan pemecahan DNA yang ketara dengan peningkatan dalam cara bergantung kepada masa. Terdapat 0.4 unit (OD) ($p < 0.05$) peningkatan dalam aktiviti caspase-3. Girinimbine juga mencetuskan pengumpulan sel-sel dalam fasa $G_0/G_1$

(kira-kira 7.5% ($p < 0.05$) berbanding dengan mengawal sel-sel) dalam perkembangan kitaran sel HepG2. Tahap intraselular spesies oksigen reaktif (ROS) dalam HepG2 sel-sel meningkat bergantung kepada masa rawatan girinimbine. Tahap awal ROS di HepG2 sel-sel 105.20 ± 5.26% telah mencapai 132.70 ± 6.63% ($p < 0.05$) selepas 3 jam rawatan. Bagi antioksidan intraselular, tahap GSH selepas permulaan peningkatan 30% menurun kepada 20% selepas 3 jam rawatan girinimbine. Kerosakan mitokondria juga telah meningkat dengan rawatan girinimbine seperti kehilangan potensi membran mitokondria dalam analisis aliran sitometrik. Kehilangan potensi membran mitokondria untuk sel-sel kawalan adalah 4.30 ± 0.21%, manakala selepas rawatan girinimbine potensi membran mitokondria menjadi 25.30 ± 1.26% pada jam yang ketiga. Kaedah Comet menunjukkan peningkatan 5 kali ganda yang signifikan ($P < 0.05$) berbanding dengan kawalan apabila didedahan kepada 100 μM girinimbine selepas 3 jam pengeraman. Girinimbine juga mendorong HSP 70 dan HSP 90 ekpresi bergantung kepada dos sehingga kepekatan 100 μM dan masa selepas 24 jam pengeraman. Walau bagaimanapun, prarawatan asid askorbik antioksidan dan pemangkin menunjukkan penurunan tahap ROS, HSP dan kerosakan mitokondria, kerosakan oksidatif DNA, kecuali peningkatan dalam tahap GSH. Semua peristiwa-peristiwa ini berlaku pada peringkat awal (iaitu, pada 3 jam selepas rawatan girinimbine sendiri), mencadangkan mekanisme tekanan oksidatif dalam mendorong apoptosis dalam HepG2 sel-sel. Kesimpulannya, hasil kajian ini sangat menyokong hipotesis girinimbine yang membantut pertumbuhan sel-sel HepG2 melalui

induksi $G_0/G_1$ fasa tangkap dan diselesaikan dengan tekanan oksidatif apoptosis yang didorong oleh laluan mitokondria.

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I certify that a Thesis Examination Committee has met on 8-10-2010 to conduct the final examination of Suvitha Syam Mohan on his thesis entitled "Cytotoxic effect of girinimbine on HepG2 cells" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of type of degree. The members of the Supervisory Committee were as follows:

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## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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**SUVITHA SYAM MOHAN**
Date: 22 September 2011

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