UNIVERSITI TEKNOLOGI MARA

NADH DEHYDROGENASE AS A MOLECULAR TARGET FOR ARTEMISININ RELATED ANTI-MALARIA DRUG SCREENING IN A YEAST MODEL

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Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy in Science

Faculty of Applied Sciences

May 2015

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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Thesis Title	:	NADH Dehydrogenase as a Molecular Target for Artemisinin Related Anti-Malaria Drug Screening in a Yeast Model
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Date	:	May 2015

ABSTRACT

Artemisinin is currently the only effective drug against malaria. However, artemisinin-resistant *Plasmodium* had begun to emerge in many malaria endemic areas. Discovery of new anti-malarial with artemisinin-like activity had been slow as the molecular target of artemisinin was yet to be established. In addition, studies on the malaria causative agent were also hampered because *Plasmodium* was difficult to culture *in-vitro*. Therefore, this research aims to develop a reliable yeast screening system to help clarify artemisinin mode of action as well as accelerate the discovery of potential anti-malaria with artemisinin-like properties. The NADH dehydrogenase enzyme, which was coded by *NDE1* and *NDI1*, in *Saccharomyces cerevisiae* as thought to be a major molecular target for artemisinin. A yeast system was constructed in which the efflux pump regulator genes, *PDR1 and PDR3,* and either *NDE1* or *NDI1* were deleted. Absence of the *PDR1* and *PDR3* genes minimized the ability of yeast to remove the test drugs into the extracellular environment, thus the drug effect could be clearly observed. From the study, Δ*pdr1*Δ*pdr3*Δ*nde1* or Δ*pdr1*Δ*pdr3*Δ*ndi1* knock-out tolerated 12 μM artemisinin and 4 μM dihydroartemisinin in contrast to Δ*pdr1*Δ*pdr3*. Hence, Δ*pdr1*Δ*pdr3* and Δ*pdr1*Δ*pdr3*Δ*nde1* were chosen to serve as the screening panels. Several compounds were found to possess artemisinin-like activities. These included black seed oil, black pepper and mangosteen. Since NADH dehydrogenase genes in yeast were homologous to *Plasmodium NDH2* gene, it was assumed that any effect towards the yeast proteins may be reflective of a similar effect towards *Plasmodium* protein. Further validation demonstrated that the cloned *NDE1* gene partially restored the yeast susceptibility to artemisinin derivative, dihydroartemisinin. Real-time PCR revealed that the yeast with cloned *NDE1* expressed NADH dehydrogenase albeit at 32-fold lower than the wild-type. Following that, random mutation to *NDE1* gene showed that most mutation was single nucleotide deletion that altered the protein sequence to produce non-functional (due to stop codons) or missense (due to different amino acid sequence) protein to resist artemisinin derivative.

ACKNOWLEDGEMENT

Alhamdulillah. Praise only for الله The Almighty.

This work is dedicated to my beloved *Nenek*, Allahyarham Haji Morshidi bin Semuni, who inspired me to dream high. This had been Hanan's promise to you...

Life of a PhD scholar is difficult to understand unless one experienced it. It gets lonelier with each passing day. It is emotionally exhausting, more than being physically challenging. When your circles of friends are moving forward in life like getting their dream jobs, earning well or even starting a family, you are struggling in the lab, having an extension of a student's life. Near to the end of a PhD, your life is at pause, moving nowhere, the research work seems to be endless and you start to feel like an underachiever.

For me, I personally feel that it takes strong will and high perseverance to remain positive during these PhD years. Even to feel optimistic requires so much effort. The consuming nature of a PhD is at times so overwhelming to the point that it is easier to get a PhD offer than to actually complete one. I really wish that people know questions like "When are you finishing? Why does it take so long?" has no answers. I had tried my best, but somehow certain things were definitely beyond my control.

Nevertheless, walking away is never my option. I am determined to finish what I started. This will indeed be an experience that I will not forget. I guess now my thesis is worth thousands of tears rather than thousands of words (if only tears were the requirement). Therefore, I would like to begin my thanks to those who help me throughout this journey. A big thank you I convey to my supervisor, Associate Prof. Dr. Mohd Faiz Foong Abdullah and co-supervisor, Dr. Umi Marshida, for the wisdom and help given. Not forgotten, to many of my lecturers, I really appreciate all the comforting hugs, the inspiring words, the chatty-time and of course for all your help and prayers.

To my beautiful family, Addy, Ummi and Hakim, sorry that Hanan takes a longer time to complete this. I love all of you for the unconditional support, blessing, and unbelievable strength. We may not have it all together but together, we have it all. My heartfelt thanks also go to Abang for brightening my days, for the comforting words and for the laughter that keeps my spirit up. You are my friend, my fiancé, my husband and soon, a father-to-be as this PhD goes along (see how long it took?). Not forgotten, to all my friends especially Yanti Yaacob and Mohd. Zafri, may we be stronger to face the world. Last but not least, I would forever hold the gratitude in my heart for many others who directly or indirectly contributed and helped in any way possible.

Al-fatihah for Allahyarham Nor Farah Shamira...a true friend, the good listener and my trusted confidante. I really miss our times together and wish you are here.

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND

1.1.1 Malaria

Malaria is a mosquito-borne disease caused by *Plasmodium* parasite infection. In 2013, Malaysia reported nearly 4000 confirmed malaria cases out of 198 million cases globally. Malaysia is currently declared in the pre-elimination phase of malaria. Based on the World Malaria Report 2014 by World Health Organization (2015), more than 500000 deaths occurred due to malaria each year. The study conducted also revealed that statistically, 90% malaria cases mostly occurred in Africa and 78% deaths involved children under the age of five.

Malaria occurrence is high, mainly in tropical and subtropical regions compared to the other areas around the world. This is mostly due to the environmental factors such as temperature and humidity. For instance, at temperature of below 20°C, *Plasmodium falciparum* is unable to complete their growth cycle inside the mosquitoes. Thus, the infectious form of *Plasmodium*, the gametocytes, cannot be produced in order to infect the human host. However, in tropical areas, the temperature favors the growth of malaria parasite to survive and multiply.

Areas where there are frequent rainfall also create suitable breeding places for *Anopheles* mosquitoes, thus increasing the intensity of vectors to transmit malaria (Centers for Disease Control and Prevention, 2012). Nonetheless, even within the tropical regions, malaria transmission is unlikely to occur at very high altitude, during colder seasons, in deserts where humidity is scarce as well as in countries where successful malaria elimination programs are extensively executed (O'Meara, Mangeni, Steketee & Greenwood, 2010).

Figure 1.1 showed the malaria-prone areas of the world (World Health Organization, 2013). In many parts, malaria transmission is not known to arise. This was represented by the abundant grey coloured areas in the map. Meanwhile, places