UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF A HETEROLOGOUS HUMAN CYP2C9-NADPH P450 REDUCTASE SYSTEM: AN *IN VITRO* ENZYME SYSTEM FOR DRUG METABOLISM STUDY

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Candidate'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 references. This topic has not been submitted to any other academic institution or non-academic institution for attainment of any other degree or qualification.

In the event that my thesis is found to violate the above mentioned declaration, I voluntarily waive the right for conferment of degree and agree to be subjected to the disciplinary rules and regulations of Universiti Teknologi MARA.

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ABSTRACT

A heterologous system that enables rapid screening of the principal routes of metabolism of drugs, herbs, food and new chemical entities (NCE) would be of enormous benefit in research and drug development. Cytochrome P450 (CYP) 2C9 is one of the principal enzymes involved in the metabolism of many drugs such as phenytoin, warfarin, tolbutamide, celecoxib, fluoxetine and losartan. However, to date its roles in the clearance of other compounds such as local herbs *Eurycoma longifolia* Jack (Tongkat Ali) have not been reported. An *in-vitro* heterologous enzyme system was developed using *E. coli* (DH5α) to study the metabolism of these compounds. Recombinant CYP2C9 and NADPH-Cytochrome P450 reductase were co-expressed in separate but compatible plasmid to generate an active *in-vitro* drug metabolizing system. The yield of the protein expressed was at the optimum level when the culture were incubated at 30°C and harvested after 24 hour. Immunoblotting demonstrated the presence of both CYP2C9 and reductase protein with sizes approximately 55 kDa and 80 kDa respectively. The kinetic activity of the enzyme was characterized using fluorescent base Vivid® CYP450 Screening Kit. Incubation of enzymes with different concentration of BOMCC substrate was done to determine the kinetic parameters (V_{max} = 64.1 nM, K_m = 99.1 μM). Assay between CYP2C9-*Eurycoma longifolia* Jack (Tongkat Ali) was carried out to study the possibility of the inhibition effect of Tongkat Ali towards the enzyme. Presence of 20 μg and 50 μg Tongkat Ali in the reaction showed reduced enzyme activity. Higher concentration of Tongkat Ali gave higher inhibition, thus lowering the velocity of enzyme in which its k_i value is 5408.9 μM. The result of this study contributes in enhancing the drug-herb interaction database profile on the metabolism pathway and inhibitory effects of local herbs towards CYP2C9.

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CHAPTER ONE

INTRODUCTION

1.1 Overview

Cytochrome P450 (CYP450) comprises a superfamily of homeoproteins, which functions as the terminal oxidase of the mixed function oxidase system. This superfamily is divided into families and further subdivided into subfamilies according to their amino acid sequences (Nelson *et. al*, 1996). In human, the most important CYPs in drug metabolism are CYP2A6, CYP2C9, CYP2C19, CYP2D6 and CYP2E1 (Taavitsainen, 2001) but the expression of CYP enzymes varies between individuals due to some factors including genetics, environmental or some diseases (Rendic, 2002). These variations will lead to inter - individual pharmacokinetics and pharmacodynamics of drugs variations (Miners *et. al*, 1998).

Several methods have been used to study human drug metabolism, for example by using human liver microsomes or homogenates, and hepatocytes cultures. However, the first two methods have its own limitations as i) it is difficult to get donation of human liver; ii) presence of a variety of CYPs in human liver; iii) most of the CYPs have similar structure and amino acid sequence hence causing difficulties in identifying specific CYPs which is responsible for the metabolism of a particular drug, and lastly; iv) level of CYPs vary throughout the population and will contribute to assay errors in the study. The introduction of heterologous expression system has managed to