THE EFFECT OF *ABRUS PRECATORIUS* METHANOL LEAVES EXTRACT IN INHIBITING GLUCOSE ABSORPTION IN *IN VITRO* STUDY

HAFEDH AHMED ABDULLAH AL-MOALEMI

A dissertation submitted in partial fulfillment of the requirements for the award of the degree of Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

FEBRUARY 2015
Dedicated with love:

To my beloved daddy; Ahmed Abdullah Al-moalemi

To my adore mom; Najmah Omar

To my lovely wife

To my dear brothers and sisters

To my two beautiful daughters; Rawa and Rafah
ACKNOWLEDGEMENT

First and foremost, all praise to Allah the Almighty, thanks to Him for giving me the opportunity and will to finish this research and to complete this dissertation. In preparing this dissertation, I was in contact with many people, researchers, academicians, and practitioners. They have contributed towards my understanding and thoughts. In particular, I wish to express my sincere appreciation to my supervisor, Dr. Siti Pauliena Mohd Bohari, for encouragement, guidance, critics and friendship. I am also indebted to Ministry of Public Health and Population in Yemen for funding my Master study also special thanks to Universiti Teknologi Malaysia (UTM) for their assistance in supplying the relevant literatures. My sincere appreciation also extends to all my colleagues and others who have provided assistance at various occasions. Unfortunately, it is not possible to list all of them in this limited space. I am grateful to all my family members, especially my wife.

A thousand thanks also to all of the staff at Biotechnology department, Faculty of Biosciences and Medical Engineering for helping me during this research, particularly, the lab assistants.
ABSTRACT

*Abrus precatorius* leaves have been used traditionally for treatment of Type II diabetes in Malaysia. In this study, the potential of the 80% methanolic extract of *Abrus precatorius* leaves has been studied for its α-glucosidase inhibitory activity and its glucose diffusion effect. The extract of this plant at varying concentrations (6.25, 12.5, 25, 50 mg/ml) were tested using *in vitro* methods. An inhibitory α-glucosidase activity was determined spectrophotometrically in a 96-well microtiter plate. The results of α-glucosidase inhibition assay revealed that *Abrus precatorius* leaves extract showed strong inhibition (65.4% and 84.6%) at the concentrations of 25 and 50 mg/ml, respectively, and represent less inhibition (25% and 28.2%) at the concentration of 6.25 and 12.5 mg/ml respectively when compared to control. Whereas, the effects of *Abrus precatorius* leaves extract on glucose movement across the sealed dialysis tube was measured spectrophotometrically using the glucose oxidase method. The results of inhibition of glucose diffusion *in vitro* assay indicated that, *Abrus precatorius* leaves extract shows a slight inhibitory effect on glucose diffusion at the concentrations of 12.5, 25 and 50 mg/ml after 16 hours, and the concentration of 50 mg/ml showed more effect after 24 hours. Nonetheless, the plant extract did not show any statistically significant effect on glucose movement after 0, 4, 8, 12, 16, 20 and 24 hours when compared to control. Therefore, it is considered that, the concentrations higher than 50 mg/ml may show a significant inhibitory effect on glucose diffusion. Overall, these results suggest that, *Abrus precatorius* leaves extract have significant inhibitory effects on glucose absorption due to the ability of plant to inhibit intestinal α-glucosidase enzyme.
ABSTRAK

Daun *Abrus precatorius* digunakan secara tradisional di Malaysia untuk merawat penyakit diabetes jenis II. Dalam kajian ini, 80% ekstrak metanol daun *Abrus precatorius* telah dikaji untuk potensinya bagi menghalang aktiviti enzim α-glucosidase dan kesan penyerapan glukosa. Pada kepekatan yang berbeza (6.25, 12.5, 25, 50 mg/ml), ekstrak tumbuhan ini telah diuji menggunakan kaedah *in vitro*. Spektrofotometrik telah digunakan untuk mengenalpasti aktiviti pembantutan aktiviti α-glucosidase pada plat 96-microtiter. Hasil kajian aktiviti pembantutan α-glucosidase menunjukkan ekstrak *Abrus precatorius* memberi kesan yang kuat (65.4% dan 84.6%) pada kepekatan 25 dan 50 mg/ml masing-masing, dan kesan pembantutan berkurangan (25% dan 28.2%) pada kepekatan 6.25 dan 12.5 mg/ml masing-masing berbanding dengan kawalan. Manakala, kesan ekstrak *Abrus precatorius* pada pergerakan glukosa merentasi tiub dialisis telah diukur menggunakan alat spektrofotometrik dengan kaedah “glukosa oxidase”. Hasil kesan pembantutan penyerapan glukosa *in vitro* menunjukkan kesan pembantutan yang kurang pada kepekatan 12.5, 25 dan 50 mg/ml selepas 16 jam dan kesan yang lebih tinggi telah ditunjukkan pada kepekatan 50 mg/ml selepas 24 jam. Walau bagaimanapun, ekstrak tumbuhan tidak menunjukkan sebarang kesan yang berbeza secara statistiknya pada pergerakan glukosa selepas 0, 4, 8, 12, 16, 20 dan 24 jam apabila dibandingkan dengan kawalan. Oleh itu, kepekatan yang lebih tinggi daripada 50 mg/ml mungkin menunjukkan kesan yang ketara pada tindak balas pembantutan penyerapan glukosa. Berdasarkan hasil kajian ini, ekstrak daun *Abrus precatorius* mempunyai kesan yang efektif terhadap pembantutan penyerapan glukosa disebabkan oleh tumbuhan ini mempunyai keupayaan untuk menghalang aktiviti enzim α-glucosidase di dalam usus.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACKNOWLEDGEMENT</td>
<td>iv</td>
</tr>
<tr>
<td></td>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>TABLE OF CONTENTS</td>
<td>vi</td>
</tr>
<tr>
<td></td>
<td>LIST OF TABLE</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td></td>
<td>LIST OF ABBREVIATION AND SYMBOLS</td>
<td>xiii</td>
</tr>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.1 Background of the Study</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.2 Problem statement</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1.3 Objectives of the study</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1.4 Significance of the study</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1.5 Scope of the study</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>LITERATURE REVIEW</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2.1 Diabetes Mellitus</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2.1.1 Introduction</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2.1.2 Classification of Diabetes Mellitus</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2.1.2.1 Insulin-dependent Diabetes Mellitus (IDDM)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2.1.2.2 Non Insulin-dependent diabetes mellitus (NIDDM)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2.1.2.3 Gestational Diabetes</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.1.3 Symptoms of Diabetes Mellitus</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.1.4 Complications of Diabetes Mellitus</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.1.5 Diagnosis of Diabetes Mellitus</td>
<td>12</td>
</tr>
</tbody>
</table>
2.1.6 Treatment of Diabetes Mellitus

2.1.6.1 Oral Anti-Diabetic Drugs

2.2 Physiology glucose metabolism

2.2.1 Starch (Carbohydrates) digestion

2.2.2 Glucose transport

2.2.3 Regulation of blood glucose concentrations

2.3 Alpha-glucosidase inhibition assay

2.4 In vitro model of inhibition of glucose diffusion

2.4.1 Glucose oxidase method

2.5 Traditional medicine

2.5.1 Herbs

2.5.2 The use of medicinal plants for treating diabetes mellitus

2.5.3 Abrus Precatorius

3 METHODOLOGY

3.1 Materials

3.1.1 Source of plant

3.1.2 Chemicals and Reagents

3.2 Extraction procedure

3.2.1 Preparation of Abrus precatorius leaves

3.2.2 Extraction of leaves

3.3 Solutions and Buffer

3.3.1 Preparation of 1U/ml Alpha-glucosidase enzyme

3.3.2 Preparation of 1mM p-nitrophenyl-alpha-D-glucopyranoside

3.3.3 Preparation of Glucose oxidase reagent

3.3.4 Preparation of 0.15M Sodium Chloride (NaCl)

3.3.5 Preparation of 0.22M D-glucose in 0.15M Sodium chloride

3.3.6 Preparation of 0.1M Sodium Carbonate (Na₂CO₃)

3.3.7 Preparation of 50 mM phosphate buffer, PH(6.8)

3.4 Methods and techniques

3.4.1 α-glucosidase inhibition Assay

3.4.2 Effects of plant extract on glucose movement
3.4.2.1 Glucose oxidase method 35

3.5 Statistical analysis 36

4 RESULT AND DISCUSSION 37

4.1 In vitro α-glucosidase inhibition study 37

4.2 Effects of Abrus precatorius extract on glucose diffusion in in vitro 40

5 CONCLUSION 45

5.1 Conclusion 45

5.2 Future Work 46

REFERENCE 47
## LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The diagnostic criteria for diabetes and lesser degrees of impaired glucose regulation</td>
<td>14</td>
</tr>
<tr>
<td>4.1</td>
<td>The percent inhibition of α-Glucosidase enzyme by methanolic extract of <em>Abrus precatorius</em> leaves and Acarbose (positive control) at varying concentrations.</td>
<td>39</td>
</tr>
<tr>
<td>4.2</td>
<td>The effect of methanolic extract of <em>Abrus precatorius</em> leaves at varying concentrations on the diffusion of glucose out of dialysis tube through 24 h incubation period</td>
<td>43</td>
</tr>
</tbody>
</table>
# List of Figures

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Pathogenesis of Type I diabetes mellitus</td>
<td>8</td>
</tr>
<tr>
<td>2.2</td>
<td>Progressive pathogenesis of Type II diabetes mellitus</td>
<td>10</td>
</tr>
<tr>
<td>2.3</td>
<td>Process of starch digestion</td>
<td>18</td>
</tr>
<tr>
<td>2.4</td>
<td>Glucose transport from a small intestine into blood stream</td>
<td>19</td>
</tr>
<tr>
<td>2.5</td>
<td>Regulation of blood glucose concentrations</td>
<td>20</td>
</tr>
<tr>
<td>2.6</td>
<td><em>Abrus precatorius</em> plant</td>
<td>25</td>
</tr>
<tr>
<td>3.1</td>
<td>Sequential extraction of <em>Abrus precatorius</em> leaves by 80% methanol</td>
<td>29</td>
</tr>
<tr>
<td>3.2</td>
<td>80% methanolic crude extract of <em>Abrus precatorius</em> leaves after the freeze dried process</td>
<td>30</td>
</tr>
<tr>
<td>3.3</td>
<td>Diagram of lab procedures for dialysis tube</td>
<td>35</td>
</tr>
<tr>
<td>4.1</td>
<td>96-well plate containing the control negative (Blank and Test) and varying concentrations of Acarbose and <em>Abrus precatorius</em> leaves extract before incubation for 30 min</td>
<td>38</td>
</tr>
</tbody>
</table>
4.2 96-well plate containing the control negative (Blank and Test) and varying concentrations of Acarbose and Abrus precatorius leaves extract after incubation for 30 min

4.4 Glucose oxidase reagent without sample

4.5 Glucose oxidase reagent with sample, after incubation for 20 minutes
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>IDDM</td>
<td>Insulin-dependent diabetes mellitus</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non-Insulin-dependent diabetes mellitus</td>
</tr>
<tr>
<td>GDM</td>
<td>Gestational Diabetes Mellitus</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>Peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>IRS1</td>
<td>Insulin receptor substrate 1</td>
</tr>
<tr>
<td>SUR</td>
<td>Sulphonylurea receptor SUR</td>
</tr>
<tr>
<td>DKA</td>
<td>Diabetic ketoacidosis</td>
</tr>
<tr>
<td>NKHS</td>
<td>Non-ketotic hyperosmolar</td>
</tr>
<tr>
<td>SGLT 1</td>
<td>Na⁺/glucose co-transporter</td>
</tr>
<tr>
<td>SGLT 2</td>
<td>Glucose transporter 2</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>Na⁺</td>
<td>Sodium ions</td>
</tr>
<tr>
<td>PNPG</td>
<td>p-nitrophenyl-α-D-glucopyranoside</td>
</tr>
</tbody>
</table>
GOD - Glucose oxidase
POD - Peroxidase
NaCl - Sodium Chloride
Na2Co3 - Sodium Carbonate
KH2PO4 - Potassium dihydrogen phosphate
K2HPO4 - Potassium hydrogen phosphate
STD - Standard
S - Sample
B - Blank
SD - Standard deviation
°C - Degree Celcius
mL - Millilitre
mM - Millimolar
μl - Microliter
g - Gram
mg - Milligram
CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Diabetes mellitus is a common chronic disease characterised by high blood glucose levels (Hyperglycemia) with disturbances of carbohydrate, protein, and fat metabolism (West, 2000). Approximately, 285 million adults worldwide suffered from diabetes mellitus in 2010. It is anticipated that the number will increase to 439 million by 2030 (Shaw et al., 2010). The World Health Organization (WHO) reported that some 347 million people worldwide suffer from diabetes mellitus and more than 80% are from low-and middle income countries (WHO, 2013). Diabetes mellitus occurs when the pancreas fails to yield sufficient insulin, or when the body cannot use the insulin it produced efficiently (Association, 2010). The insulin hormone is responsible for decreasing the glucose levels in the blood circulation (Association, 2010).

The classification of this disease is dependent on the pathogenesis of the diabetes mellitus. The first classification of diabetes was published in 1997 by a team of experts from the American Diabetes Association. Their findings were
approved by the World Health Organization (WHO) in 1998 (Alberti et al., 1998a). In 2003, it was updated and refined by the ADA and updated again by the WHO in 2006 to the current classification which includes Insulin-dependent diabetes mellitus (IDDM), Non-Insulin-dependent diabetes mellitus (NIDDM), Gestational Diabetes Mellitus (GDM), and other specific types of diabetes (Alberti and Zimmet, 1998; Genuth et al., 2003; Organization, 2006). Among these types of diabetes mellitus, NIDDM (Type II) is a metabolic disorder characterised by hyperglycaemia, which often results from insulin resistance or β-cell dysfunction due to the interaction between environmental and genetic factors (Stumvoll et al., 2005). Diabetes mellitus, NIDDM (Type II), has been recognized as a global health problem of the 21st century, and represents 90% of all cases of diabetes mellitus (Chen et al., 2012; Scully, 2012).

The medical symptoms of diabetes mellitus are excessive thirst, hunger, muscular weakness, weight loss, and continuous urination. These symptoms were first recorded by the ancient Egyptians in the Ebers papyrus some 3500 years ago, and by the Greek physician Aretaens the Cappadocian (A.D 30-90) and Galen (A.D 130-200) (Farnsworth and Seligman, 1971). An increase in blood glucose levels for a sustained periods can lead to several complications such as kidney failure, cardiovascular diseases, hepatic, nerve damage, and blindness (Chen, et al., 2012; Hussain et al., 2008). Diagnosis of diabetes is dependent on the demonstration of increased concentrations of glucose in the blood. This can be determined through the use of the oral glucose tolerance test which has been used as a diagnostic criteria (Association, 2008).

Treatment of diabetes mellitus aims to reduce levels of blood glucose to normal or close to normal in the patients. One of the most effective ways to control Type II diabetes is to reduce glucose absorption through inhibiting carbohydrate digestion, which is an important source for glucose in the human body. Digestion of carbohydrate occurs in the gastrointestinal tract, and requires two enzymes, pancreatic α-amylase and intestinal α-glucosidases to release absorbable glucose (Jones et al., 2011).
The function of α-glucosidases enzymes is by breaking down the disaccharides that come from food or produced through digestion of carbohydrate by α-amylases to the monosaccharides (glucose) (Lieberman and Marks, 2009). The inhibition of α-glucosidase can help delay the digestion of carbohydrates in the intestine thereby better managing the Type II diabetes (Heacock et al., 2005). Current medications of α-glucosidase inhibitors are comparatively expensive and have toxic side-effects such as acute hypoglycemia at higher doses, liver problems, diarrhea, nausea, lactic acidosis, and weight gain (Avery et al., 2008; Bastaki, 2005). This has encouraged people to use plants and herbs as alternative source of α-glucosidase inhibitors because it is more acceptable and cheaper (Kee et al., 2013).

There are many dietary supplements of plant origins, which have been studied and have therapeutic potential agents for the treatment of diabetes and its complications (Mohamed et al., 2012). One of the local herbs in Malaysia, locally called Akar Saga, scientifically known as Abrus precatorius, is used as an antidiabetic remedy (Monago and Alumanah, 2005, Bhatia et al., 2013). Abrus precatorius is a common herb belonging to the Fabaceae family and is widely available in the forests of Malaysia, Indonesia, India, and Nigeria to name a few (Monago and Alumanah, 2005, Bhatia et al., 2013). Several studies have reported that Abrus precatorius has a wide range of medical effects (Bhatia et al., 2013).

In this study, the potential of the 80% methanolic extract of Abrus precatorius leaves has been studied for its α-glucosidase inhibition and its glucose diffusion effect. The extract of these leaves at different concentrations were tested using in vitro methods. Inhibitory α-glucosidase activities have been determined spectrophotometrically in a 96-well Microtiter plate. Whereas, the effects of Abrus precatorius leaves extract on glucose movement across the sealed dialysis tube have been measured spectrophotometrically using the glucose oxidase assay.
1.2 Problem statement

Alpha-glucosidase is involved in the breakdown of complex carbohydrate molecules to glucose in the small intestine before they can be absorbed into the bloodstream. Patients with Type II diabetes suffer from an increase in blood glucose levels after eating complex carbohydrates. The inhibition of the glucose absorption through inhibiting α-glucosidase or inhibiting glucose transport would help to reduce the impact of rising postprandial blood glucose and better manage Type II diabetes. In the current study, the potential of the *Abrus precatorius* plant methanolic extract has been studied for its α-glucosidase inhibiting and its effect on glucose diffusion.

1.3 Objectives of the study

i. To study the potential of *Abrus precatorius* plant methanolic extract as α-glucosidase inhibitors.

ii. To investigate the glucose entrapment study by using *Abrus precatorius* plant methanolic extract.

1.4 Significance of the study

In this study, the potential of *Abrus precatorius* leaves methanolic extract to inhibit α-glucosidase and glucose diffusion has been studied *in vitro* by using α-
glucosidase inhibitor assay and the glucose entrapment assay. This helps to provide a natural source of the glucose absorption inhibitors that will eventually have an impact on the treatment and management of Type II diabetes.

1.5 Scope of the study

The *Abrus precatorius* leaves methanolic extract at different concentrations were tested using *in vitro* methods for their anti-diabetic effects on α-glucosidase inhibition and glucose diffusion. Inhibitory α-glucosidase activities have been determined spectrophotometrically in a 96-well Microtiter plate. Whereas, the effects of *Abrus precatorius* leaves extract on glucose movement across the sealed dialysis tube have been measured spectrophotometrically using the glucose oxidase method.
REFERENCES


activity of the methanolic extract from Tournefortia hartwegiana: An anti-

antioxidative activity of phenolic and flavonoids compounds extracted from seeds
of Abrus precatorius. *International Journal of Pharmacy and Pharmaceutical
Sciences*, 1(2), 136-140.

glucose entrapment and alpha-glucosidase inhibition of mucilaginous substances

Park, M.-H., Ju, J.-W., Park, M. and Han, J. (2013). Daidzein inhibits carbohydrate
digestive enzymes in vitro and alleviates postprandial hyperglycemia in diabetic

Wilkins.


group as a protecting/activating group for 2-acetamido-2-deoxyglucose.
*Carbohydrate research*, 338(5), 455-458.

Royal Society of Chemistry.


