ANTIDIABETIC ACTIVITIES OF OIL PALM (*Elaeis guineensis* Jacq.)
FRUIT AND PALM OIL MILL EFFLUENT EXTRACTS

By

MOHD FAEZ BIN SHARIF

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

October 2014
COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ANTIDIABETIC ACTIVITIES OF OIL PALM (*Elaeis guineensis* Jacq.) FRUIT AND PALM OIL MILL EFFLUENT EXTRACTS

By

MOHD FAEZ BIN SHARIF

October 2014

Chairperson: Associate Professor Muhajir Hamid, PhD
Faculty: Biotechnology and Biomolecular Sciences

Diabetes is one of the top ten causes of death in Malaysia. According to the fourth National Health and Morbidity survey conducted in 2011, it is estimated that 15.2% (2.6 million) of Malaysians adults 18 years old and above suffer from diabetes. Most antidiabetic drugs available are associated with several side effects which explain the current prevalence of diabetes. Therefore researches are needed in exploring the new alternative for antidiabetic treatment which are safe, efficient and exert a lesser amount of side effects. Recently the oil palm, *Elaeis guineensis* has been explored in several antidiabetic studies. The oil palm leaves have been found to reduced hyperglycemia in STZ-induced diabetic rats due to the high polyphenolic content. Nevertheless several parts of oil palm which are also contained high amount of polyphenol such as fruit and the effluent from palm oil processing, POME are never been investigated in antidiabetic study. Therefore, this study was conducted to evaluate the antidiabetic properties of oil palm fruit and POME extracts through the *in vivo* antihyperglycemic evaluation. In addition, an initiative was made to study the possible mechanisms involve using *in vitro* models. Raw POME and oil palm fruit were subjected to solvent extraction using ethanol. The extracts collected were further used in the *in vivo* and *in vitro* experiments. To evaluate the antihyperglycemic property of both extracts in diabetic rats, the rats were given the extracts orally using intragastric gavage in the *in vivo* study. The *in vitro* models were design to evaluate the potential antidiabetic mechanisms involve by using the glucose uptake, insulin secretion as well as adiponectin secretion model. The results had shown that acute treatment of POME and oil palm fruit extracts reduced fasting and postprandial hyperglycemia in streptozotocin-induced diabetic rats. Following 28-days treatment, both extracts at concentration of 500 mg kg\(^{-1}\) b.w significantly reduced hyperglycemia, improved the body weight and increased insulin secretion in streptozotocin induced diabetes rats. Through *in vitro* evaluation, the oil palm fruit extract (500µg ml\(^{-1}\)) were found to stimulate the insulin secretion from BRIN BD11 cell line the most. Moreover both extracts also enhanced basal and insulin mediated glucose uptake into adipocytes, muscles and liver cells. In the evaluation of adiponectin secreting activity, the POME extracts significantly increased adiponectin secretion in both basal and insulin-stimulated state. However the oil palm fruit extracts significantly increased adiponectin secretion only under the insulin stimulated state. The HPLC analysis had shown the presence of gallic acid and
catechin as part of the bioactive compound for both extracts. In conclusion, from in vivo evaluation the treatment of POME and oil palm fruit extracts were shown to reduce hyperglycemia at different prandial state in diabetic rats. Both extracts also did not cause severe hypoglycemia in normal rats. The in vitro study suggested that the antihyperglycemic property of POME and oil palm fruits were mediated through insulin secretion from pancreatic β-cells, enhancement of glucose uptake by the muscles, adipocytes and liver cells and amplification of adiponectin secretion from adipocytes cells. Therefore various antihyperglycemic potential of both extracts together with its property that did not cause hypoglycemia make them suitable to be develop as new oral antidiabetic drugs.
Abstrak tesis yang dikenalkan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

AKTIVITI ANTIDIABETIS EKSTRAK BUAH KELAPA SAWIT (Eleais guineensis Jacq.) DAN SISA EFLUEN KILANG MINYAK SAWIT

Oleh

MOHD FAEZ BIN SHARIF

Oktober 2014

Pengerusi: Muhajir Hamid, PhD
Fakulti: Bioteknologi dan Sains Biomolekul

ACKNOWLEDGEMENTS

First and foremost, all thanks to Allah the Al Mighty, for granting me the chance and ability to successfully complete this study. In preparing this thesis, many people have contributed their advices and knowledge towards my understanding and thoughts. This page is specially designed to express my appreciation to those peoples.

I wish to express my deepest gratitude to my dearest supervisor, Assoc. Prof. Dr. Muhajir Hamid and my co-supervisor Prof. Dr. Amin ismail and Dr. Zainah Adam for the great guidance, encouragements, efforts and valuable advice given throughout the study. Special thanks also to my dearest wife Siti Nurhafizah Binti Abu Samah for all the support and helps during this journey.

Not to be forgotten, my dearest parents, brother and sister whom had support and pray for my success in completing this study. I am also indebted to all lectures of Faculty of Biotechnology and Biomolecular Sciences, lab assistants and fellow friends for their guidance, advice and motivation. Without their continued support and interest, this thesis would not have been the same as presented here.
I certify that a Thesis Examination Committee has met on 13th October 2014 to conduct the final examination of Mohd Faez Bin Sharif on his thesis entitled “Antidiabetic activities of oil palm (*Elaeis guineensis* Jacq.) Fruit and Palm Oil Mill Effluent extracts” in accordance with the Universities and University Colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

**Assoc. Prof. Dr. Janna Ong Abdullah, PhD**
Associate professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairperson)

**Dr. Syahida Ahmad, PhD**
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Prof. Dr. Md Zuki Abu Bakar, PhD**
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

**Dr. Asna Urooj, PhD**
Lecturer
Department of Studies in Food Science & Nutrition
Universiti of Mysore
India
(External Examiner)

---

**ZULKARNAIN ZAINAL, PhD**
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 26 February 2015
I certify that a Thesis Examination Committee has met on 13 Oktober 2014 to conduct the final examination of Mohd Faez bin Sharif on his thesis entitled "Antidiabetic Activities of Oil Palm (Elaeis guineensis Jacq.) Fruit and Palm Oil Mill Effluent Extracts" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Janna Ong binti Abdullah, PhD**  
Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Chairman)

**Md Zuki bin Abu Bakar @ Zakaria, PhD**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Internal Examiner)

**Syahida binti Ahmad, PhD**  
Senior Lecturer  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Asna Urooj, PhD**  
Senior Lecturer  
Department of Studies in Food Science & Nutrition  
University of Mysore  
(External Examiner)

---

**ZULKARNAIN ZAINAL, PhD**  
Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 26 February 2015
Declaration by graduate student

I hereby confirm that:

• this thesis is my original work;
• quotations, illustration and citations have been duly referenced;
• this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
• intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
• written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
• there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: ________________  Date: 20 March 2015

Name and Matric No.: Mohd Faez Bin Sharif, GS 28635
Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: ____________________________ Signature: ____________________________
Name of Chairman of Member of
Supervisory Supervisory
Committee: Associate Prof. Dr. Muhajir Committee: Dr. Zainah Adam
Hamid

Signature: ____________________________
Name of Member of
Supervisory Committee:
Prof. Dr. Amin Ismail
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ABSTRACT</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xviii</td>
</tr>
</tbody>
</table>

## CHAPTER

### 1 GENERAL INTRODUCTION
1.1 Background of study 1
1.2 Statement of problems 1
1.3 Justification of study 2
1.4 Hypothesis of study 3
1.5 Objectives of study 3

### 2 LITERATURE REVIEW
2.1 Diabetes mellitus 4
2.2 Type 1 diabetes mellitus 4
2.3 Type 2 diabetes mellitus 5
2.4 Treatment of diabetes mellitus 5
2.4.1 Treatment for type 1 diabetes mellitus 5
2.4.2 Treatment for type 2 diabetes mellitus 6
2.4.2.1 Sulfonylureas (SUs) 6
2.4.2.2 Biguanide 6
2.4.2.3 Thiazolidinediones (TZDs) 7
2.4.2.4 α-glucosidase inhibitors 7
2.4.2.5 Meglitinide 8
2.5 Blood glucose level (BGL) regulation in human 8
2.5.1 Stimulation of insulin secretion from pancreatic β-cells 9
2.5.2 Enhancement of glucose uptake into peripheral Cells 9
2.5.3 Augmentation of adiponectin secretion from adipocytes cells 10
2.6 Models for antidiabetic research 11
2.6.1 In vivo models 11
2.6.2 In vitro models 12
2.7 Plants as alternative antidiabetic agents 13
2.8 Oil palm (Elaeis guineensis) 13
2.9 Nutritional properties of palm oil and its component 15
2.10 Palm oil mill effluent (POME) 16
3 EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITIES OF *E. GUINEENSIS* FRUITS AND POME EXTRACTS IN STZ-INDUCED DIABETIC RATS

3.1 Introduction 18
3.2 Materials and methods 19
    3.2.1 Samples collection 19
    3.2.2 Sample preparation and extraction procedures 19
    3.2.3 Experimental animals and diabetes induction 19
    3.2.4 Hypoglycemic evaluation in normal rats and antihyperglycemic evaluation in fasting and post-prandial diabetic rats 20
    3.2.5 Oral glucose tolerance test (OGTT) 20
    3.2.6 Evaluation of long term treatment in STZ-induced diabetic rats 21
    3.2.7 Statistical analyses 21

3.3 Results 22
    3.3.1 The hypoglycemic activity of POME and OPF extract in normal rats 22
    3.3.2 Antihyperglycemic activity of POME and OPF extract in STZ-induced diabetic rats at fasting state. 24
    3.3.3 Antihyperglycemic activity of POME and OPF extract in STZ-induced diabetic rats at postprandial state. 26
    3.3.4 Effect of POME extract on oral glucose tolerance test (OGTT) of STZ-induced diabetic rats 28
    3.3.5 Effect of OPF extract on oral glucose tolerance test (OGTT) of STZ-induced diabetic rats 29
    3.3.6 Effect of long term four weeks POME treatment on rat’s body weight 30
    3.3.7 Effect of long term four weeks OPF treatment on rat’s body weight 31
    3.3.8 Effect of long term four weeks POME treatment on blood glucose level in normal and STZ-induced diabetic rats 32
    3.3.9 Effect of long term four weeks OPF treatment on blood glucose level in normal and STZ-induced diabetic rats 33
    3.3.10 Effect of long term four weeks POME and OPF treatment on rat’s plasma insulin level 34

3.4 Discussion 35
3.5 Conclusion 39

4 EVALUATION OF *E. GUINEENSIS* FRUITS AND POME EXTRACTS ON CELL VIABILITY AND ADIPONECTIN SECRETION FROM 3T3F442A ADIPOCYTES

4.1 Introduction 40
4.2 Materials and methods 42
    4.2.1 Sample preparation and extraction procedures 42
    4.2.2 Cell line 42
    4.2.3 Cell culture 42
4.2.4 Cell viability assay
4.2.5 Examination of POME and OPF extracts on the secretion of adiponectin from 3T3F442A adipocytes
4.2.6 Determination of adiponectin concentration
4.2.7 Statistical analyses

4.3 Results
4.3.1 Effects of POME and OPF extracts on the viability of BRIN BD11 cells
4.3.2 Effects of POME and OPF extracts on the viability of Chang liver cells
4.3.3 Effects of POME and OPF extracts on the viability of L6 myotubes
4.3.4 Effects of POME and OPF extracts on the viability of 3T3F442A adipocytes
4.3.5 Effect of OPF and POME extracts on stimulating adiponectin secretion from 3T3F442A adipocytes

4.4 Discussions
4.5 Conclusion

5 EFFECT OF ELAEIS GUINEENSIS FRUITS AND POME EXTRACTS ON GLUCOSE UPTAKE ACTIVITY IN L6 MYOTUBES, 3T3F442A ADIPOCYTES AND CHANG LIVER CELLS
5.1 Introduction
5.2 Materials and methods
5.2.1 Sample preparation and extraction procedure
5.2.2 Cell line
5.2.3 Cell line maintenance
5.2.4 Glucose uptake activity of POME and OPF extracts in L6 myotubes
5.2.5 Glucose uptake activity of POME and OPF extracts in 3T3F442A adipocytes
5.2.6 Glucose uptake activity of POME and OPF extracts in Chang liver cells
5.2.7 Statistical analyses
5.3 Results
5.3.1 Glucose uptake activity of POME and OPF extracts in L6 myotubes
5.3.2 Glucose uptake activity of POME and OPF extracts in 3T3F442A adipocytes
5.3.3 Glucose uptake activity of POME and OPF extracts in Chang liver cells
5.4 Discussions
5.5 Conclusion
6 EVALUATION OF INSULIN SECRETING ACTIVITY FROM BRIN BD11 CELL AND SCREENING OF POLYPHENOL COMPOUNDS IN OIL PALM FRUIT AND POME EXTRACTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

6.1 Introduction 77

6.2 Materials and methods 79
6.2.1 Sample preparation and extraction procedures 79
6.2.2 Cell line 79
6.2.3 Examination on insulin secreting activity of BRIN BD11 in the presence of POME and OPF extracts 80
6.2.4 Determination of insulin concentration 80
6.2.5 Determination of insulin secretion mechanisms 80
6.2.6 Screening of polyphenolic compounds in POME and OPF extracts using High Performance Liquid Chromatography (HPLC) 80
6.2.7 Statistical analyses 80

6.3 Results 81
6.3.1 Effect of POME and OPF extracts on insulin secreting activity of BRIN BD11 cell line 81
6.3.2 Effect of various insulin modulators on insulin secretion from BRIN BD11 cell line 83
6.3.3 Screening of POME and OPF extracts by HPLC 84

6.4 Discussions 87
6.5 Conclusion 90

7 SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH
7.1 Summary and general conclusion 91
7.2 Recommendation for future research 93

REFERENCES 95
APPENDICES 108
BIODATA OF STUDENT 134
LIST OF PUBLICATION 135
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Hypoglycemic effect of POME and OPF extracts on fasting blood glucose in normal rats</td>
<td>23</td>
</tr>
<tr>
<td>3.2</td>
<td>Antihyperglycemic effect of POME and OPF extracts on fasting blood glucose level in STZ-induced diabetic rats</td>
<td>25</td>
</tr>
<tr>
<td>3.3</td>
<td>Antihyperglycemic effect of POME and OPF extracts on postprandial blood glucose level in STZ-induced diabetic rats</td>
<td>27</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Harvested oil palm fruit from a 7 years old oil palm tree</td>
<td>14</td>
</tr>
<tr>
<td>2.2</td>
<td>Palm oil mill effluent (POME)</td>
<td>17</td>
</tr>
<tr>
<td>3.1</td>
<td>Oral glucose tolerance tests of experimental rats treated with POME extract</td>
<td>28</td>
</tr>
<tr>
<td>3.2</td>
<td>Oral glucose tolerance tests of experimental rats treated OPF extract</td>
<td>29</td>
</tr>
<tr>
<td>3.3</td>
<td>Changes in rat’s body weight during long term four weeks POME treatment</td>
<td>30</td>
</tr>
<tr>
<td>3.4</td>
<td>Changes in rat’s body weight during long term four weeks OPF treatment</td>
<td>31</td>
</tr>
<tr>
<td>3.5</td>
<td>Changes in rat’s BGL during long term four weeks POME Treatment</td>
<td>32</td>
</tr>
<tr>
<td>3.6</td>
<td>Changes in rat’s BGL during long term four weeks OPF Treatment</td>
<td>33</td>
</tr>
<tr>
<td>3.7</td>
<td>Changes in rat’s plasma insulin level during long term four weeks treatment</td>
<td>34</td>
</tr>
<tr>
<td>4.1</td>
<td>Cells viability of BRIN BD11 cells after 72 hours exposure to POME extract</td>
<td>45</td>
</tr>
<tr>
<td>4.2</td>
<td>Cells viability of BRIN BD11 cells after 72 hours exposure to OPF extract</td>
<td>46</td>
</tr>
<tr>
<td>4.3</td>
<td>Cells viability of BRIN BD11 cells after 72 hours exposure to glybenclamide</td>
<td>46</td>
</tr>
<tr>
<td>4.4</td>
<td>Cells viability of Chang liver cells after 72 hours exposure to POME extract</td>
<td>47</td>
</tr>
<tr>
<td>4.5</td>
<td>Cells viability of Chang liver cells after 72 hours exposure to OPF extract</td>
<td>48</td>
</tr>
<tr>
<td>4.6</td>
<td>Cells viability of Chang liver cells after 72 hours exposure to rosiglitazone maleate</td>
<td>48</td>
</tr>
<tr>
<td>4.7</td>
<td>Cells viability of L6 myotubes after 72 hours exposure to POME extract</td>
<td>49</td>
</tr>
</tbody>
</table>
4.8 Cells viability of L6 myotubes after 72 hours exposure to OPF extract

4.9 Cells viability of L6 myotubes after 72 hours exposure to Metformin

4.10 Cells viability of 3T3F442A after 72 hours exposure to POME extract

4.11 Cells viability of 3T3F442A after 72 hours exposure to OPF extract

4.12 Cells viability of 3T3F442A after 72 hours exposure to rosiglitazone maleate

4.13 Effect of OPF extract on basal and insulin-stimulated adiponectin secretion from 3T3F442A adipocytes

4.14 Effect of POME extract on basal and insulin-stimulated adiponectin secretion from 3T3F442A adipocytes

4.15 Effect of rosiglitazone maleate on basal and insulin-stimulated adiponectin secretion from 3T3F442A adipocytes

5.1 Effect of POME extract on basal and insulin-mediated glucose uptake activity of L6 myotubes

5.2 Effect of OPF extract on basal and insulin-mediated glucose uptake activity of L6 myotubes

5.3 Effect of metformin on basal and insulin-mediated glucose uptake activity of L6 myotubes

5.4 Effect of POME extract on basal and insulin-mediated glucose uptake activity of 3T3F442A adipocytes

5.5 Effect of OPF extract on basal and insulin-mediated glucose uptake activity of 3T3F442A adipocytes

5.6 Effect of rosiglitazone maleate on basal and insulin-mediated glucose uptake activity of 3T3F442A adipocytes

5.7 Effect of POME extract on basal and insulin-mediated glucose uptake activity of Chang liver cells

5.8 Effect of OPF extract on basal and insulin-mediated glucose uptake activity of Chang liver cells

5.9 Effect of rosiglitazone maleate on basal and
insulin-mediated glucose uptake activity of Chang liver cells

6.1 Effect of POME extract on insulin secretion from BRIN BD11 cells

6.2 Effect of OPF extract on insulin secretion from BRIN BD11 cells

6.3 Effect of glibenclamide on insulin secretion from BRIN BD11 cells

6.4 Effect of various insulin secretion modulators on the insulin secretion activity of 500 µg/ml OPF from BRIN BD11 cells

6.5 Chromatogram of POME extract

6.6 Chromatogram of OPF extract

6.7 Gallic acid

6.8 Catechin
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACUC</td>
<td>Animal Care and Use Committee</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;Gluose&lt;/sub&gt;</td>
<td>Area under the glucose curve</td>
</tr>
<tr>
<td>BGL</td>
<td>Blood glucose level</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CaCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Calcium chloride</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified eagle’s medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>FBG</td>
<td>Fasting blood glucose</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>FPG</td>
<td>Fasting plasma glucose</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>Water</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Sulfuric acid</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid</td>
</tr>
<tr>
<td>IBMX</td>
<td>3-isobutyl-1-methylxanthine</td>
</tr>
<tr>
<td>IDDM</td>
<td>Insulin dependent diabetes mellitus</td>
</tr>
<tr>
<td>K*&lt;sub&gt;ATP&lt;/sub&gt; channel</td>
<td>ATP-sensitive potassium channel</td>
</tr>
<tr>
<td>K</td>
<td>Potassium</td>
</tr>
<tr>
<td>KCl</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>KRB</td>
<td>Krebs Ringer Bicarbonate buffer</td>
</tr>
<tr>
<td>KH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Potassium dihydrogen phosphate</td>
</tr>
<tr>
<td>M&lt;sub&gt;g&lt;/sub&gt;S&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Magnesium sulphate</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4,5-Dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium Bromide</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NaHCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Sodium hydrogen carbonate</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non-insulin dependent diabetes mellitus</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>OPF</td>
<td>Oil palm fruit extract</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>POME</td>
<td>Palm oil mill effluent</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozotocin</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>TZDs</td>
<td>Thiazolidinidiones</td>
</tr>
<tr>
<td>TRIS</td>
<td>2-Amino-2-hydroxymethyl-propane-1,3-diol</td>
</tr>
<tr>
<td>UPM</td>
<td>Universiti Putra Malaysia</td>
</tr>
<tr>
<td>USA</td>
<td>United state of America</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>centimeter cubic</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>µl</td>
<td>microlitres</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>Symbol</td>
<td>Unit Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>µM</td>
<td>micromolar</td>
</tr>
<tr>
<td>mM</td>
<td>millimolar</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>µm</td>
<td>micrometer</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter</td>
</tr>
<tr>
<td>m</td>
<td>meter</td>
</tr>
<tr>
<td>rpm</td>
<td>round per minute</td>
</tr>
<tr>
<td>α</td>
<td>Alpha</td>
</tr>
<tr>
<td>β</td>
<td>Beta</td>
</tr>
<tr>
<td>γ</td>
<td>Gamma</td>
</tr>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>ºC</td>
<td>Degree celcius</td>
</tr>
</tbody>
</table>
CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of Study

Diabetes mellitus which characterized by persistent hyperglycemia in blood is a chronic disease that is relatively common throughout the world. It is resulted from defects of insulin secretion or insulin action and can also be cause by combination of both factors (Alberti and Zimmet, 1998). It is normally accompanied by increased risk to oxidative stress, hypertension and severe atherosclerosis (Reusch, 2003).

Diabetes is one of the top ten causes of death in Malaysia. According to the fourth National Health and Morbidity survey conducted in 2011, it is estimated that 15.2% (2.6 million) of Malaysians adults 18 years old and above suffer from diabetes. The amount has bypassed the assumption made by World Health Organization (WHO) which estimated that in the year of 2030, Malaysia would have a total of 2.48 millions diabetes sufferers. This indicates that Malaysia is having a faster increase rate of diabetic disease. The rapid rising trend in the prevalence of diabetes could possibly due to the urbanization, changes in dietary habits, growth of population and inactive lifestyle (Zanariah et al., 2008).

Poor glycemic controls in diabetes patient will always contribute to the prevalence of diabetes complications such as retinopathy, neuropathy, and albuminuria. The records from the Ministry of Health (MOH) showed that the number of diabetic patients admitted to hospitals had increased from 19, 629 cases in 1991 to 30, 661 in 2001. It shows about 56% increments over 10 years. Diabetes mortality rates also increased from 254 death in 1991 to 380 in 2001 (Ooyub et al., 2004). This indicates that diabetes had become major problem in Malaysia. Therefore a stepped up efforts are required in controlling and preventing this chronic disease.

1.2 Statement of Problems

Diabetes can be classified into two class which are type I and type II diabetes mellitus. In type I diabetes, the maintenance of hyperglycemia is limited to insulin therapy (Jacobson et al., 2009). Different types of insulin analogues are available for this purpose such as Humalog, NovoRapid, Levimir and Lantus. Those analogues were categorized according to their times of action onset and duration. On the other hand the regulation of hyperglycemia in type II diabetes patient were mostly by changes of diet, active lifestyle, regular exercise and the uses of oral antidiabetic drugs. Antidiabetic drugs for the treatment of type II diabetes mellitus vary from sulfonylureas, biguanides, thiazolidinediones, \( \alpha \)-glucosidase inhibitor, amylin synthetic derivatives and incretin mimetics. The drugs were categorized into different groups based on its antidiabetic mechanisms and mode of action (Chehade and Mooradian, 2000).
The major problem liaises with the conventional antidiabetic drugs were the limitations of the drugs itself. The drugs can bring up undesirable adverse effects such as vomiting, weight gain, nausea and diarrhoea. Furthermore most conventional antidiabetic drugs were also expensive making them unavailable and unaffordable especially in the third world and developing countries where it is difficult to have access to those modern and high cost drugs (Babu et al., 2007). The clinical trial also were still lacking for most antidiabetic drugs making them less efficient (Kirchheiner et al., 2005). For instance, drugs from sulfonylureas group was reported to cause hypoglycemia and weight gain. There are also reports on occurring of death due to prolonged severe hypoglycemia. Metformin on the other hand always associated with gastrointestinal discomfort, diarrhea, and nausea. The α-glucosidase inhibitor also can cause gastrointestinal disturbance, lethal ileus and renal tumors. Meanwhile, drugs from the thiazolidinediones groups can cause fluid retention, liver injury and anemia (Bell, 2002).

Due to the limitations of conventional antidiabetic drugs, searching for a new alternative antidiabetic agent for diabetes treatment is needed. It is crucial to find antidiabetic agents that are safe and efficient to replace the conventional antidiabetic drugs to overcome the limitations brought by those drugs.

1.3 Justification of Study

Palm oil mill effluent (POME) is a waste generated from the oil palm manufacturing process. The POME is often discarded in disposal ponds, resulting in the leaching of contaminants that pollute the groundwater and soil while releasing methane gas to the atmosphere. Tan and colleague reported in 2001 that water soluble phytochemicals from the palm fruit mesocarp which partition into aqueous phase during the oil palm manufacturing process could contains several phenolic compounds. This includes gallic, protocatechuic, gentisic, chlorogenic, coumaric, ferulic, and caffeic acids, as well as hesperidin and catechins (Tan et al., 2001). Recent studies have evaluated the potential of phytochemicals from oil palm in treating various diseases. Oil palm phenolics have been reported previously to inhibit proliferation of estrogen-receptor-positive human breast adenocarcinoma cells, human lung carcinoma cells (Shamala et al., 2010) and promoting vascular relaxation (Mahinda et al., 2002). Furthermore recent study by Rosalina and colleague has revealed the potential of oil palm leaves extract in reducing hyperglycemia and lipid oxidation in STZ-rats (Rosalina et al., 2011). Therefore it is a great effort to evaluate the POME as a new source of oil palm phenolics to treat diabetes. The POME is cheaper and also help reducing environmental pollution as the waste is being utilized.

Phenolic compounds are often found in plants. It comprises of groups such as the phenolic acids, flavonoids and tannins. Several publications on the antioxidative activities of plant derived phenolics have been reported previously (Wang and Ballington, 2007; Materska and Perucka, 2005). Phenolic acids have attracted special attention lately due to its strong inhibitory activity on oxidation induced by peroxyl radicals (Hu and Kitts, 2001). Oil palm fruit is also a rich source of water soluble-phenolics antioxidants like any other fruits. Plants with high phenolics antioxidant
compounds exert high potential as supplements for improving blood glucose control and preventing long-term complications in diabetics (Gallegher et al., 2003). Many research on the antioxidative capacity of the oil palm fruit have been conducted (Neo et al., 2010; Nagendran et al., 2005). Despite the high antioxidant activity of oil palm reported previously however the studies on its potential as alternative antidiabetic agent are never been studied. Therefore this study is conducted to evaluate the potential of oil palm fruit as a new antidiabetic agent to combat diabetes mellitus.

1.4 Hypothesis of Study

I. Treatment of oil palm fruit and POME extracts reduce hyperglycemia in STZ-induced diabetic rats and has no severe hypoglycemia effect in normal rats
II. improve insulin secretion activity of BRIN BD11 cell line
III. improve glucose uptake activity into L6 myotubes, 3T3F442A adipocytes and Chang liver cells and
IV. improve adiponectin secretion activity of 3T3F442A adipocytes.

1.5 Objectives of study

The main objective of this study was to evaluate the antidiabetic properties of oil palm (Elaeis guineensis) fruit and POME extract. The specific objectives of this study were:
1. To evaluate the hypoglycaemic and antihyperglycaemic activity of oil palm fruit and POME extracts in normal and STZ-induced diabetic rats.
2. To evaluate the toxicity of oil palm fruit and POME extracts and their effect on adiponectin secretion from adipocyte cells.
3. To investigate the effect of oil palm fruit and POME extract on glucose uptake into muscle, adipocyte and liver cell.
4. To evaluate the effect of oil palm fruit and POME extract on insulin secretion from pancreatic beta cells and to identify the phenolics compound that exists in both extracts.
REFERENCES


