UNIVERSITI PUTRA MALAYSIA

HYPOGLYCEAMIC, HYPOLIPIDEMIC AND HISTOPATHOLOGICAL EFFECTS OF BROWN SEAWEED (*SARGASSUM POLYCYSTUM C. AGARDH*) EXTRACTS ON TYPE 2 DIABETIC RATS

MAHSA MOTESHAKERI

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BY

MAHSA MOTESHAKERI

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

August 2011

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Dedicated to my beloved mum and dad, brothers and sister
….. For their patience and support

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

HYPOGLYCEAMIC, HYPOLIPIDEMIC AND HISTOPATHOLOGICAL EFFECTS OF BROWN SEAWEED (*SARGASSUM POLYCYSTUM C. AGARDH*) EXTRACTS ON TYPE 2 DIABETIC RATS

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MAHSA MOTESHAKERI

August 2011

Chairman: Suhaila Bt Mohamed, PhD

Faculty: Food Science and Technology

Diabetes mellitus is a serious global problem that is a major cause of disability and hospitalization in the world. This disorder is characterized by hyperglycemia and hyperlipidemia that are implicated in the development of microvascular and macrovascular complications. Recently, seaweeds have been the center of focus as a natural source of biological compounds useful for human health. Brown seaweed, *Sargassum polycystum* has been reported to be rich in biologically active substances with potential healing effects. In a preliminary study *S. polycystum* polar extract exhibited hypoglycemic and hypolipidemic effects on streptozotocin-induced diabetic rats. The present study

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was carried out to investigate hypoglycemic and hypolipidemic effects of seaweed ethanolic and water extracts in type 2 diabetes rat model. Male *Sprague-Dawley* rats (weighing 200–250 g) were divided into seven groups: normal control (NC) group fed with standard rodent diet (SRD) till the end of experiment, diabetic control (DC) group fed high-sugar, high-fat diet (HSHFD) for 16 weeks and then induced with 35 mg/kg body weight of streptozotocin (STZ), diabetic rats treated with 300 mg ethanolic extract/kg body weight (DE300), diabetic rats treated with 150 mg ethanolic extract/ kg body weight (DE150), diabetic rats treated with 300 mg water extract/ kg body weight (DW300), diabetic rats treated with 150 mg water extract/ kg body weight (DW150), and diabetic rats treated with 250 mg metformin/ kg body weight (DM). Diabetes was induced in all diabetic groups by feeding rats with daily fresh prepared HSHFD for 16 weeks and then injection of low dose streptozotocin. Body weight, blood glucose level and serum lipid parameters were measured in 16 weeks. The HSHFD-fed rats exhibited significant increase ($P<0.05$) in body weight (BW) over time while significant increase ($P<0.05$) was also observed in NC group. Low-density lipoprotein-cholesterol (LDL-C) increased significantly ($P<0.05$) in HSHFD-fed group as compared to NC group. High-density lipoprotein-cholesterol (HDL-C) and HDL-C/TC ratio decreased significantly ($P<0.05$) in HSHFD-fed group as compared to normal control

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group. However, fasting blood glucose, triglyceride (TG) and total cholesterol (TC) levels did not change significantly ($P>0.05$) as compared to normal control group. Besides, homeostasis model assessment of insulin resistance (HOMA-IR) showed significantly higher ($P<0.05$) insulin resistance index in HSHFD-fed rats as compared to normal control group. Furthermore, after 2 hours of glucose challenge, the rats in the HSHFD-fed group all presented higher ($P<0.05$) blood glucose concentration than did the NC group. The HSHFD-fed rats presented a fasting plasma insulin that was significantly ($P<0.05$) higher than that of NC group in intraperitoneal glucose tolerance test (IPGTT). At the end of 22 days of seaweed treatment DC, DW300 and DE300 exhibited significantly ($P<0.05$) lower body weight than NC group. Fasting blood glucose levels (BGL) were significantly ($P<0.05$) reduced in all diabetic treated groups except in DW150 group. DW300 appeared to be more insulinotropic but it was not significant ($P>0.05$) compared to DC, while treatment with metformin significantly ($P<0.05$) increased insulin level. There was a significant reduction ($P<0.05$) in the percentage of HbA1c in the DE300, DE150, DW300 and DM groups compared to DC group. All seaweed extracts reduced total cholesterol and triglyceride level significantly ($P<0.05$) compared to DC group. LDL-C level was significantly ($P<0.05$) reduced in DW300 group compared to DC group. HDL-C/TC ratio was significantly ($P<0.05$) improved in DE150, DW300, DW150 and DM groups

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compared to DC group. Pancreas histopathological results showed significantly ($P<0.05$) lower lesions in all treated groups compared with DC group. Moreover, regeneration of islets of Langerhans with more intact appearance was found in DE300, DW300 and DM groups compared with DC, DE150 and DW150 groups. However, DE150, DW150 and DM groups showed fewer lesions in the liver and kidney tissues compared to DC, DE300 and DW300 groups. High doses of both extracts have more hypoglycemic properties with better regeneration effects on pancreas than low doses. However, 150 mg/kg of seaweed ethanolic and water extracts caused fewer side effects on the liver and kidney tissues as compared to the high doses (300 mg/kg). *Sargassum polycystum* extracts showed good hypoglycemic and hypolipidemic effects with pancreas regeneration properties, hence may be potentially useful for the prevention of metabolic syndrome. It is preferably to be used at low dosage of about 150 mg/kg body weight, since higher dosage may cause some liver or kidney lesions.

Keywords: *Sargassum polycystum,* Brown seaweed, Type 2 diabetes, Hypoglycemic, Hypolipidemic, *Sprague-Dawley*.

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

HIPOGLIKEMIK, HIPOLIPIDEMIK DAN HISTOPATOLOGI KESAN EKSTRAK RUMPAI LAUT PADA (*SARGASSUM POLYCYSTUM C. AGARDH*) DALAM JENIS 2 DIABETIK

oleh

MAHSA MOTESHAKERI

Ogos 2011

Pengerusi: Suhaila Bt Mohamed, PhD

Fakulti: Sains Dan Technologi Makanan

Diabetes adalah masalah global yang sangat serius dan merupakan penyebab utama kehilangan upaya dan kes yang memerlukan rawatan hospital. Hiperglikemia dan hiperlipidemia dicirikan deh komplikasi mikrovaskular dan makrovaskular. Sejak kebelakangan ini, rumpai laut telah menjadi fokus utama sebagai sumber semulajadi bahan aktif biologi kerana ia mempunyai nilai kesihatan yang berguna. Rumpai laut perang (*Sargassum polysystum*) dilaporkan kaya dengan bahan aktif biologi yang mempunyai potensi memulihkan kesihatan dengan baik. Kajian awal menunjukkan ekstrak polar *S. polycystum* mempunyai kesan hipoglikemia dan hipolipidemia terhadap tikus diabetes

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induksi Streptozotocin. Kajian ini dijalankan untuk menyelidik kesan rumpai laut etanolik dan ekstrak air di dalam model tikus diabetes jenis 2 ke atas hiperglikemia dan hiperlipidemia. Tikus jantan jenis *Sprague-Dawley* (dengan berat badan 200-250 g) telah dibahagikan kepada 7 kumpulan; kumpulan kawalan normal yang diberi makan diet kawalan (SRD) hingga ke akhir eksperimen, kumpulan kawalan diabetes tikus yang diberi makan diet kandungan gula and lemak tinggi, selama 16 miggu dan kemudiannya diinduksikan dengan 35 mg/kg streptozotocin (STZ), kumpulan eksperimen tikus diabetes diberikan ektstrak etanol (DE300, 300 mg/kg), tikus diabetes diberikan ekstrak etanol (DE150, 150 mg/kg), tikus diabetes yang diberikan ekstrak air (DW150, 150 mg/kg), tikus diabetes yang diberikan bersama metformin (DM, 250 mg/kg). Diabetes diinduksikan dengan pemenerimaan diet HSHF (4.6% kcal/g) yang disediakan segar setiap hari selama 16 minggu dan kemudian disuntik dengan 35 mg/kg streptozotocin. Berat badan, tahap glukosa darah dan parameter lipid serum diukur 16 minggu selepas rawatan HSHFD. Tikus-tikus yang diberi makan HSHFD mempamerkan peningkatan yang signifikan ($P<0.05$) di dalam berat badan berbanding kumpulan kawalan dimana berat badan bertambah secara signifikan ($P>0.05$) di dalam kumpulan HSHF secara berperingkat. Kolesterol lipoprotein berkepadatan rendah (HDL-C) dan HDL-C/TC berkurangan secara signifikan ($P>0.05$) berbanding dengan

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kumpulan kawalan normal. Walaubagaimanapun, glukosa plasma ketika berpuasa , trigliserida (TG) dan tahap jumlah kolesterol tidak berubah secara signifikannya (P>0.05) berbanding dengan kumpulan kawalan normal. Selain daripada itu, tahap rintangan insulin secara signifikannya (P<0.05) lebih tinggi dalam tikus yang diberi makan HSHFD berbanding kumpulan kawalan normal. Tikus HSHFD menunjukkan konsentrasi glukosa darah yang tinggi (P<0.05) daripada kumpulan yang diberi SRD. Tikus-tikus HSHFD telah menunjukkan plasma puasa insulin yang signifikan (P<0.05) tinggi daripada tikus SRD di dalam ujian toleransi intraperitoneal glukosa selepas hiperglikemik induksi oleh suntikan STZ di dalam induksi insulin ketahanan tikus-tikus bergula dan berlemak tinggi. Tikus-tikus diabetes terbahagi kepada dua kumpulan rawatan (DC, DE300, DE150, DW300, DW150, DM) dan kesan perubatan rumpai laut ke atas tikus diabetes jenis 2 telah diuji di dalam kesemua tujuh kumpulan. Keputusan penyelidikan selepas 22 hari menerima rawatan rumpai laut mempamerkan penurunan berat badan yang signifikan (P<0.05) di dalam kumpulan DC, DW300 dan DE300. Tahap glukosa darah berpuasa berkurangam di dalam kesemua kelompok kumpulan rawatan diabetes secara signifikan kecuali kumpulan DW150. DW300 muncul menjadi lebih peka insulin tetapi tidak signifikan (P>0.05) berbanding DC, selain itu rawatan bersama metformin meningkatkan tahap insulin secara signifikan (P<0.05). Terdapat juga

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pengurangan signifikan (P<0.05) didalam peratusan HbA1C di dalam kumpulan DE300, DW300 dan DM berbanding dengan kumpulan DC. Kesemua kumpulan rawatan ekstrak rumpai laut telah mengurangkan jumlah kolesterol dan tahap triglyceride secara signifikan berbanding kumpulan DC. Tahap LDL-C telah berkurangan secara secara signifikan (P<0.05) di dalam kumpulan DW300 berbanding kumpulan DC. Nisbah HDL-C/TC bertambah baik pada kesemua kumpulan perlakuan tetapi di dalam kumpulan DE150, DW300, DW150 dan DM ia adalah lebih signifikan (P<0.05) berbanding dengan kumpulan DC. Keputusan ujian histopatologi pankreas menunjukkan pemulihan islets of Langerhans yang lebih baik didalam kumpulan DE300, DW300 dan DM berbanding dengan kumpulan-kumpulan DC, DE150, dan DW150. Kumpulan DE150, DW150 dan DM menunjukkan sedikit lesi di dalam tisu hati dan buah pinggang berbanding kumpulan-kumpulan DC, DE300 dan DW300. Dos yang tinggi untuk kedua-dua ekstrak mempunyai lebih banyak kesan hipoglikemik dan regenerasi yang lebih baik terhadap pankreas berbanding dos yang rendah. Walaubagaimanapun, 150 mg/kg rumpai laut etanolik dan ekstrak air menyebabkan sedikit kesan sampingan terhadap tisu hati dan buah pinggang berbanding dos yang tinggi (300 mg/kg). Ekstrak *Sargassum Polysystum* menunjukkan kesan baik pada hipoglikemik, hipolipidemik dan anti-kegemukan dengan wujudnya komponen regenerasi pancreas. Ia berpotensi

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untuk mencegah sindrom metabolik dan sebaiknya digunakan pada dos yang rendah (150 mg/kg berat badan) dan dos yang tinggi akan menyebabkan kerosakan hati dan buah pinggang.

Keywords: *Sargassum polycystum,* Rumpai laut perang, jenis 2 diabetes, Hipoglikemik, Hipolipidemik, *Sprague-Dawley*.

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Sincerely
Mahsa Moteshakeri

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I certify that an Examination Committee has met on ..... to conduct the final examination of **Mahsa Moteshakeri** on her thesis entitled "**Hypoglycemic, hypolipidemic and histopathological effects of brown seaweed (*Sargassum polycystum C. Agardh*) extracts on type 2 diabetic rats**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysa [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows.

……..
……
Universiti Putra Malaysia
(Chairman)

……..
…….
Universiti Putra Malaysia
(Internal Examiner)

……..
……..

Universiti Putra Malaysia
(Internal Examiner)

…….
……
……..
(External Examiner)

BUJANG BIN KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:……

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This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfillment of requirement for degree of **Master of Science**. The members of the Supervisory Committee were as follows:

Suhaila Bt Mohamed, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Mohd Hair Bejo, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Boo Huey Chern, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:……

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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 other degree at Universiti Putra Malaysia or other institution.

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Date: 19 August 2011

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