UNIVERSITI PUTRA MALAYSIA

TAMARINDUS INDICA L. PULP AQUEOUS EXTRACT EFFECTS IN HIGH FAT DIET-INDUCED OBESE SPRAGUE-DAWLEY RATS

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By

KHAIRUNNUUR FAIRUZ AZMAN

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

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

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Chairman: Zulkhairi Haji Amom, PhD

Faculty : Medicine and Health Sciences

The present study addressed the anti-obesity, hypolipemic and antioxidative effects of the crude aqueous extract of the pulp fruit of *Tamarindus indica* L. on diet-induced obese rats *in vivo*, as well as the extract's antioxidant action and toxicity potential, *in vitro*. *In vitro* antioxidative properties of the *T. indica* extract were assessed via DPPH free radical scavenging assay, ferric-reducing antioxidant power (FRAP) assay and Folin-Ciocalteau method. Toxicity potential of the extract was determined *in vitro* via brine shrimp lethality test (BSLT). To assess its *in vivo* anti-obesity, hypolipemic and antioxidative effects, *T. indica* extract of various doses (5 mg/kg, 25 mg/kg and 50 mg/kg) were administered to prior high-fat diet induced obese rats via force-feeding once daily for 10 weeks. Blood sample was withdrawn via cardiac puncture under diethyl ether anesthesia. The blood sampling was performed five times of five weeks interval for each rat.

In vitro, the *T. indica* extract presented radical scavenging ability, as assessed by DPPH and FRAP assays. A strong correlation was observed between the phenolics concentration of the extracts and its antioxidant potency which indicated that the phenolics content was likely to contribute to the antioxidant activity of the extracts. Brine shrimp lethality test (BSLT) results showed that the *T. indica* extract was virtually non-toxic to the shrimps.

In vivo, the *T. indica* extract was proven to have weight reduction and hypolipemic effects in which the group that was given the extract had significantly lower ($p<0.05$) body weight, plasma lipid profile (cholesterol, low density lipoprotein and triglyceride), insulin, glucose and leptin levels in compare to the untreated obese group. The anti-obesity action of *T. indica* extract is possibly via lowering of plasma fatty acid synthase (FAS) levels. The extract was also proven to possess antioxidative effects; indicated by decreased MDA levels and increased Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) enzymes activities.

The supplementation of *T. indica* extract to the experimental animals was proven to be safe and non-toxic to the animals' liver indicated by the reversal of the liver enzymes (Aspartate Transaminase, Alkaline Phosphatase, Alanine Transaminase and Gamma-glutamyltransferase) levels (which was increased earlier prior to high-fat diet induction) to be almost similar with the normal control group. Histological study of the liver showed *hepatic steatosis* state in the diet-induced obese rats. The administration of the *T. indica* extract caused amelioration of fatty liver and significant decrease ($p<0.05$) of adipose tissue weights than that of the untreated obese group. In conclusion, this study

indicates the potential of *T. indica* pulp extract as anti-obesity, hypolipemic and antioxidative agent.

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

KESAN EKSTRAK AKUEUS BUAH *TAMARINDUS INDICA* L. KE ATAS TIKUS SPRAGUE-DAWLEY YANG TERARUH OBESITI SECARA DIET TINGGI LEMAK

Oleh

KHAIRUNNUUR FAIRUZ AZMAN

Jun 2010

Pengerusi: Zulkhairi Haji Amom, PhD

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Kajian ini mengetengahkan kesan anti-obesiti, hipolipemik dan antioksidatif ekstrak berair buah *Tamarindus indica* L. Ekstrak ini telah diuji pada tikus yang teraruh obesiti secara *in vivo*. Aktiviti antioksidan ekstrak *T. indica* diuji secara *in vitro* dengan menggunakan asai DPPH, asai FRAP dan kaedah Folin-Ciocalteau. Potensi keracunan ekstrak diuji secara *in vitro* melalui ujian keracunan anak udang (BSLT). Untuk mengkaji kesan anti-obesiti, hipolipemik dan antioksidatif secara *in vivo*, ekstrak *T. indica* pada tiga dos (5 mg/kg, 25 mg/kg dan 50 mg/kg) diberikan kepada tikus yang telah diaruh obesiti menggunakan diet tinggi lemak. Ekstrak diberikan secara paksaan oral sekali sehari selama 10 minggu. Sampel darah diambil melalui tusukan jantung setelah tikus dibius menggunakan dietil eter. Sampel darah setiap tikus diambil sebanyak lima kali setiap lima minggu.

In vitro, ekstrak *T. indica* menunjukkan kebolehan meneutralkan radikal bebas apabila diuji dengan asai DPPH dan FRAP. Terdapat korelasi yang kuat antara kepekatan fenolik ekstrak dan potensi antioksidannya yang menunjukkan bahawa kandungan fenolik inilah yang menyumbang kepada aktiviti antioksidan ekstrak. Keputusan ujian BSLT menunjukkan bahawa ekstrak *T. indica* tidak toksik kepada udang yang diuji.

In vivo, ekstrak *T. indica* telah dibuktikan berkesan menurunkan berat badan dan kesan hipolipemik dimana kumpulan yang telah diberikan ekstrak menunjukkan berat badan, aras profil lemak (kolesterol, lipoprotein ketumpatan rendah dan trigliserida) plasma dan aras leptin yang lebih rendah secara signifikan berbanding kumpulan obes yang tidak dirawat. Kesan anti-obesiti ekstrak *T. indica* ini berkemungkinan disebabkan oleh kebolehannya menurunkan aras enzim asid lemak sintase (FAS) plasma. Ekstrak *T. indica* juga dibuktikan mempunyai kesan antioksidatif; yang ditunjukkan oleh pengurangan kepekatan MDA dan peningkatan aktiviti enzim Superoksida Dismutase (SOD) dan Glutatione Peroksida (GPx).

Supplementasi ekstrak *T. indica* kepada haiwan eksperimen dibuktikan adalah selamat dan tidak toksik kepada hepar haiwan tersebut yang ditunjukkan oleh pengurangan semula aras enzim hepar (Aspartate Transaminase, Alkaline Fosfatase, Alanine Transaminase and Gamma-glutamiltransferase) (yang sebelumnya meningkat disebabkan oleh aruhan diet tinggi lemak) ke aras yang setara dengan kumpulan kawalan normal. Kajian histologi pada hepar menunjukkan keadaan '*hepatic steatosis*' pada tikus yang teraruh obesiti. Pemberian ekstrak *T. indica* menyebabkan pembaikpulihan hepar berlemak dan penurunan signifikan berat tisu adipos berbanding kumpulan obes yang

tidak diberikan sebarang rawatan. Kesimpulannya, kajian ini menunjukkan potensi ekstrak *T. indica* sebagai agen anti-obesiti, hipolipemik dan antioksidatif.

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I certify that an Examination Committee has met on 21st June 2010 to conduct the final examination of Khairunnuur Fairuz Azman on her Master of Science thesis entitled "*Tamarindus indica* L. (Asam Jawa) Pulp Aqueous Extract and Its Effects In High-Fat-Diet-Induced Obese Sprague-Dawley Rats" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee were as follows:

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

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Date: 21 October 2010

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 to any other degree at Universiti Putra Malaysia or at any other institution.

KHAIRUNNUUR FAIRUZ AZMAN

Date: 21 June 2010

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