

**EFFICACY OF *CENTELLA ASIATICA* IN REDUCING
OXIDATIVE STRESS IN HYDROGEN PEROXIDE-
INDUCED *SPRAGUE DAWLEY* RATS**

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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To my beloved husband, families and friends.....

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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Faculty : Food Science and Technology

Centella asiatica is one of the local medicinal plants that have been claimed to have various medicinal effects. The study was designed to investigate the effect of *Centella asiatica* extract and powder on blood lipid profile, blood lipid peroxidation and antioxidant enzymes activity in oxidative stress induced male *Sprague Dawley* rats. In the first phase of the study, the experimental rats were fed with 0.3% (w/w) *Centella asiatica* extract, 1.5% (w/w) *Centella asiatica* powder, 5.0% (w/w) *Centella asiatica* powder and 0.3% (w/w) α -tocopherol for 6 weeks. Oxidative stress in these rats was induced by giving them 0.03% (v/v) hydrogen peroxide (H_2O_2) in drinking water. Normal rats were provided with commercial Gold Coin diet. Malonaldehyde (MDA) level and activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) before and during treatments were monitored. Histopathological examination of selected organs was done at the end of the study. The results showed that MDA level was significantly ($p < 0.05$) higher in the normal rats compared to others. Dietary supplementation of *Centella asiatica* (extract and powder) and α -tocopherol significantly ($p < 0.05$) reduced lipid peroxidation in the experimental rats. However, there was no significant differences in the activities of antioxidant enzymes and histopathology observations of the organs of the rats.

H_2O_2 (0.05%, 0.10% and 0.15%) were given to rats via drinking water for 6 weeks in determining effective H_2O_2 level in inducing oxidative stress in the experimental rats. At the end of the study, the experimental rats were sacrificed and organs namely heart, liver and kidney were weighed and fat

content of the organs was determined. Results showed that rats treated with 0.10% (v/v) and 0.15% (v/v) H₂O₂ had significantly (p<0.05) increased MDA level compared to that of normal and 0.05% (v/v) H₂O₂ treated rats. The finding suggested that supplementation of 0.10% (v/v) and 0.15% (v/v) H₂O₂ are sufficient in inducing oxidative stress in the experimental rats.

Second phase of the study was conducted using increase level of H₂O₂ and prolong duration of the treatments. Experimental rats were fed with 0.3% (w/w) *Centella asiatica* extract, 5.0% (w/w) *Centella asiatica* powder and 0.3% (w/w) α -tocopherol for 25 weeks. Oxidative stress was induced by giving the rats 0.1% (v/v) H₂O₂ in drinking water. Normal rats were provided with commercial Gold Coin diet. Blood lipid profile [cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triacylglycerol (TG)], MDA, CAT and SOD activities in experimental rats were monitored throughout the treatments. Histopathological examination of the heart, liver and kidney of the rats was done after the treatments ended. Results of the study indicated that *Centella asiatica* extract and powder significantly (p<0.05) increased serum HDL and cholesterol level compared to normal rats. *Centella asiatica* extract and powder also significantly lowered serum TG and LDL concentrations compared to normal rats at the end of the study. Results also showed lowered MDA levels in experimental rats consuming *Centella asiatica* extract, powder and α -tocopherol. The decrease in the activity of SOD and increase in CAT activity in *Centella asiatica* and α -tocopherol supplemented rats as compared to normal rats appeared to be the response towards decreased in free radical formation and prevention of H₂O₂ accumulation respectively. Histopathological examination of the heart, liver and kidney revealed no obvious changes in *Centella asiatica*-fed rats. The results suggested that long-term consumption of *Centella asiatica* extract and powder is not toxic to heart, liver or kidney. Results also revealed that *Centella asiatica* extract and powder may ameliorate H₂O₂-induced oxidative stress by increasing HDL concentration, decreasing the level of TG, LDL, lipid peroxidation and altering antioxidant defense system in experimental rats. These effects may be attributed to the antioxidant components and polyphenol substances present in *Centella asiatica*.

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

KEMUJARABAN *Centella asiatica* MENURUNKAN STRES OKSIDATIF PADA TIKUS *SPRAGUE DAWLEY* YANG DIRANGSANG DENGAN HIDROGEN PEROKSIDA

Oleh

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Oktober 2005

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Centella asiatica adalah sejenis tumbuhan ubatan tempatan yang telah didakwa mempunyai pelbagai kesan perubatan. Kajian dijalankan untuk menyelidik kesan ekstrak dan serbuk *Centella asiatica* terhadap profil lemak darah, pengoksidaan lemak darah dan aktiviti enzim antioksidan dalam tikus jantan *Sprague Dawley* yang telah dirangsang stres oksidatif. Di dalam fasa pertama kajian, tikus ujikaji telah diberi 0.3% (berat/berat) ekstrak *Centella asiatica*, 1.5% (berat/berat) dan 5.0% (berat/berat) serbuk *Centella asiatica* dan 0.3% (berat/berat) α -tokoferol selama 6 minggu. Stres oksidatif pada tikus ini telah dirangsang dengan memberikan 0.03% (isipadu/isipadu) hidrogen peroksida (H_2O_2) dalam air minuman. Tikus normal telah dibekalkan dengan diet komersial Gold Coin. Aras malonaldehyde (MDA) dan aktiviti katalase (CAT), superoksid dismutase (SOD) dan glutathion peroksida (GPx) sebelum dan semasa rawatan telah dipantau. Ujian histopatologi organ terpilih telah dilakukan di akhir kajian. Keputusan menunjukkan bahawa aras MDA tikus-tikus normal lebih tinggi berbanding dengan tikus ujikaji yang lain. Penambahan *Centella asiatica* (ekstrak dan serbuk) dan α -tokoferol telah mengurangkan pengoksidaan lemak dengan signifikan ($p < 0.05$) pada tikus ujikaji. Walaubagaimanapun tiada perubahan yang signifikan dapat dilihat pada aktiviti enzim antioksidan dan pemerhatian histopatologi organ tikus.

H_2O_2 (0.05%, 0.10% dan 0.15%) dalam air minuman telah diberi pada tikus ujikaji selama 6 minggu untuk menentukan peratus H_2O_2 yang berkesan dalam merangsangkan stres oksidatif. Pada akhir kajian, tikus telah dikorbankan dan organ seperti jantung, hati dan buah pinggang ditimbang dan

kandungan lemak dalam organ tersebut ditentukan. Keputusan menunjukkan bahawa tikus ujikaji yang dirawat dengan 0.10% (isipadu/isipadu) dan 0.15% H₂O₂ (isipadu/isipadu) telah mengalami peningkatan aras MDA dengan signifikan ($p < 0.05$) berbanding dengan tikus normal dan tikus yang diberi 0.05% H₂O₂. Keputusan menunjukkan bahawa penambahan 0.10% dan 0.15% H₂O₂ adalah berkesan merangsangkan stres oksidatif pada tikus yang dikaji.

Fasa kedua kajian dijalankan dengan penggunaan aras H₂O₂ yang lebih tinggi dan tempoh rawatan yang lebih panjang. Tikus yang dikaji telah diberi 0.3% ekstrak *Centella asiatica*, 5.0% serbuk *Centella asiatica* dan 0.3% α -tokoferol selama 25 minggu. Stres oksidatif telah dirangsang dengan pemberian 0.1% H₂O₂ dalam air minuman tikus. Tikus normal telah dibekalkan dengan diet komersial Gold Coin. Profil lemak darah [kolesterol, lipoprotein berketumpatan tinggi (HDL), lipoprotein berketumpatan rendah (LDL) dan triasilgliserol (TG)], MDA, aktiviti CAT dan SOD di dalam tikus telah diawasi sepanjang rawatan. Pemeriksaan histopatologi terhadap organ jantung, hati dan buah pinggang tikus terbabat telah juga dilakukan selepas rawatan ditamatkan. Keputusan kajian menunjukkan peningkatan aras serum HDL dan kolesterol dengan signifikan ($p < 0.05$) tikus yang diberi ekstrak dan serbuk *Centella asiatica* berbanding dengan tikus normal. Ekstrak dan serbuk *Centella asiatica* juga didapati merendahkan kepekatan serum TG dan LDL secara signifikan ($p < 0.05$) tikus yang dikaji berbanding dengan tikus normal di akhir kajian. Kajian juga menunjukkan penurunan aras MDA pada tikus yang memakan ekstrak dan serbuk *Centella asiatica*; dan α -tokoferol. Penurunan aktiviti SOD dan peningkatan aktiviti CAT di dalam tikus yang diberi makan *Centella asiatica* dan α -tokoferol berbanding dengan tikus normal, telah menampakkan reaksi terhadap pengurangan pembentukan radikal bebas dan pencegahan pengumpulan H₂O₂ pada tikus ini. Pemeriksaan histopatologi terhadap jantung, hati dan buah pinggang mendedahkan bahawa tiada perubahan yang ketara pada tikus yang diberi makan *Centella asiatica*. Keputusan mencadangkan bahawa ekstrak dan serbuk *Centella asiatica* yang dimakan dalam jangka masa yang panjang didapati tidak toksik terhadap jantung, hati atau buah pinggang. Keputusan juga menunjukkan bahawa ekstrak dan serbuk *Centella asiatica* boleh menurunkan stres oksidatif yang dirangsang oleh kehadiran H₂O₂ dengan meningkatkan kepekatan HDL, menurunkan aras TG, LDL, pengoksidaan lemak dan mengubah sistem pertahanan antioksidan di dalam tikus yang dikaji. Kesan ini berkemungkinan merujuk kepada komponen antioksidan dan bahan polifenol yang terdapat di dalam *Centella asiatica*.

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I certify that an Examination Committee met on 12 October 2005 to conduct the final examination of Mahanom Binti Hussin on her Master of Science thesis entitled “Efficacy of *Centella asiatica* in Reducing Oxidative Stress in Hydrogen Peroxide Induced *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 candidate be awarded the relevant degree. Members of the Examination Committee are as follows :

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

MAHANOM BINTI HUSSIN

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