

Effect of *Centella asiatica* extract and powder on hydrogen peroxide-treated *Sprague-Dawley* rats

[Kesan jangka pendek ekstrak dan serbuk pegaga (*Centella asiatica*) terhadap tikus *Sprague-Dawley* yang dirawat dengan hidrogen peroksida]

H. Mahanom*, A.H. Azizah**, M. Suhaila**, S. Nazamid**, I. Maznah*** and B. Mohd. Hair****

Keywords: *Centella asiatica*, lipid peroxidation, malonaldehyde, hydrogen peroxide, *Sprague-Dawley* rats

Abstract

A study was conducted to investigate the effect of *Centella asiatica* extract and powder on dietary intake, body weight, organ weights and blood lipid peroxidation of hydrogen peroxide (H₂O₂)-treated male *Sprague-Dawley* rats. The experimental rats were fed with 0.3% (w/w) *C. asiatica* extract, 1.5% (w/w) *C. asiatica* powder, 5.0% (w/w) *C. asiatica* powder and 0.3% (w/w) α -tocopherol for 6 weeks. To induce oxidative stress, the rats were given drinking water spiked with 0.03% (v/v) H₂O₂. The amount of dietary intake, organ and body weight, and malonaldehyde (MDA) level throughout the study 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 treated rats. Dietary supplementation of *C. asiatica* (extract and powder) and α -tocopherol significantly ($p < 0.05$) reduced lipid peroxidation in the experimental rats. However, there were no significant differences in dietary intake and histopathology observations of the organs of the rats.

Introduction

Centella asiatica or pegaga is one of the local medicinal plants that has been claimed to have various medicinal effects (Kartnig 1988). This medicinal plant and its preparations have been in use since ancient times especially in the Ayurvedic medical

system of India and in the folk medicine of China and Madagascar (Jaganath and Teik 2000). *Centella asiatica* is also consumed to a limited extent as processed product such as tea, juice and as pharmaceutical products like pills and capsules. In traditional medicine, *C. asiatica* has been used for

*Food Technology Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia

**Department of Food Science, Faculty of Food Science and Technology, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

***Laboratory of Molecular Biomedicine, Institute of Bioscience, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

****Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Authors' full names: Mahanom Hussin, Azizah Abd. Hamid, Suhaila Mohamed, Nazamid Saari, Maznah Ismail and Mohd. Hair Bejo

E-mail: mahanom@mardi.gov.my

©Malaysia Agricultural Research and Development Institute 2011

treating bronchitis, asthma, excessive secretion of gastric juices, dysentery, leucorrhoea, kidney trouble, urethritis and dropsy in many communities (Burkill 1966; Goh et al. 1995). This herb is said to have a direct action on lowering the blood pressure and is often referred to as a rejuvenating medicament (Jayaweera 1982). Previous phytochemical investigations on *C. asiatica* had led to the isolation of biologically active triterpenoids (Singh and Rastogi 1968; Luo and Jin 1981; Sahu et al. 1989; Gunther and Wagner 1996), phenolic compounds (Zainol et al. 2003; Mohamad-Khairi 2004) and other chemical constituents (Kartnig 1988). Recent studies also discovered that the phenolic compounds present were highly responsible for the antioxidant activity in *C. asiatica* and the activity was found to be as good as that of α -tocopherol at the same concentration tested (Abdul-Hamid et al. 2002; Zainol et al. 2003).

The role of reactive oxygen species (ROS) has been implicated to be the causative factor involved in many human degenerative diseases of aging and antioxidants have been found to have some preventive and therapeutic effects on these diseases (Ames et al. 1993). Hydrogen peroxide (H_2O_2), one of the main ROS, has been demonstrated to cause lipid peroxidation and DNA oxidative damage in cells (Halliwell and Aruoma 1991). Multiple papers have described high levels of H_2O_2 as being cytotoxic to a wide range of animals, plants and bacterial cells in culture (Hampton and Orrenius 1997; Clement et al. 1998; Halliwell and Gutteridge 1999). The neurotoxicity of H_2O_2 is exerted mainly by formation of the highly reactive $OH\bullet$, although depletion of reduced glutathione (GSH) levels, and the secondary rupture of calcium homeostasis, can also contribute to toxic effects of H_2O_2 (Farber et al. 1990; Rimpler et al. 1999). Various studies have shown the potential effect of H_2O_2 as one of the ROS that can induce oxidative stress in cell line model (Fallarero et al. 2003; Hsieh et al. 2004).

Oxidative stress occurs in living organisms when the production of ROS exceeds the ability to prevent their accumulation by the antioxidant defense system, employing enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). In view of the role of ROS in the etiology of a number of chronic diseases, considerable efforts have been directed towards the discovery of effective antioxidants that can impede lipid peroxidation (Aruoma 1996). The efficacy of such compounds is derived primarily from their ability to prevent the accumulation of toxic by-products such as malonaldehyde (MDA) produced during lipid peroxidation. Thus, the present study was designed to investigate the effect of *C. asiatica* extract and powder on H_2O_2 treated Sprague-Dawley rats.

Materials and methods

Plant materials

Centella asiatica extract and powder used in this study were obtained and prepared by the Forest Research Institute of Malaysia (FRIM). In general, extraction and drying method of *C. asiatica* are given in Figures 1 and 2 since the detail methods are considered as intellectual properties of FRIM.

Chemicals and reagents

All chemicals used were of the highest purity grade. Ethylenediaminetetraacetic acid (EDTA), nicotinamide adenine dinucleotide phosphate (NADPH), thiobarbituric acid (TBA), nitrobluetetrazolium (NBT), glutathione, glutathione reductase, Triton X-100, riboflavin, bovine serum albumin and Folin Ciocalteu reagent were purchased from Sigma Chemical Co. St. Louis, Missouri. Hydrogen peroxide (H_2O_2 , 30%), hydrochloric acid (HCl, 36.5–38.0%) and petroleum ether were obtained from J.T. Baker Inc. Philipsburg, USA. Trichloroacetic acid (TCA) was obtained from Fisher Scientific (Hong Kong) Ltd. All other reagents used were of analytical grade.

Preparation of treatment diets

Standard laboratory diet (Gold Coin commercial rat's feed) in powder form was mixed with *C. asiatica* extract, *C. asiatica* powder and α -tocopherol in order to prepare the treatment diets. Flow chart for the treatment diet preparations is given in Figure 3.

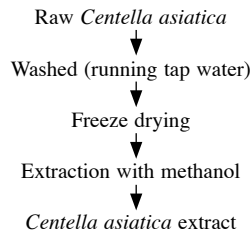


Figure 1. Preparation of *Centella asiatica* extract

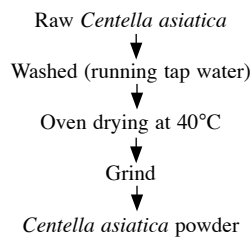


Figure 2. Preparation of *Centella asiatica* powder

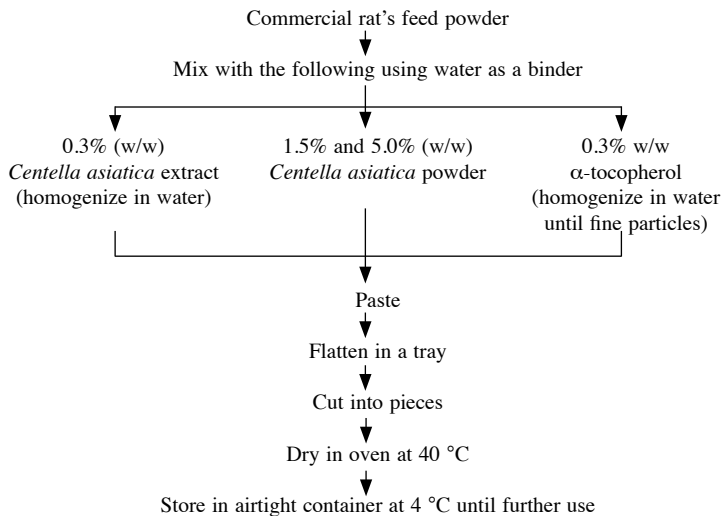


Figure 3. Preparation of treatment diets

Feeding rats with treatment diets

A total of 24 adult male *Sprague-Dawley* rats, aged approximately 3 months old, and weighing in the range of 300–500 g, were used in the study. The rats were housed in separate cages (61 cm x 41 cm x 44.5 cm in size) and kept in a cabin at 20 ± 1 °C, with a 12 h light-dark cycle. They were provided with a standard laboratory diet (Gold Coin commercial rat feed) as a normal diet, treatment diet (*C. asiatica* extract and powder; α -tocopherol) and water *ad libitum* with free access to it. Rats were randomly divided into six groups of four rats and treated for 6 weeks as follows: (1) normal diet; (2) normal diet + 0.03% (v/v) H₂O₂; (3) normal diet + 0.03% (v/v) H₂O₂ + 0.3% (w/w) *C. asiatica* extract; (4) normal diet + 0.03% (v/v) H₂O₂ + 1.5% (w/w) *C. asiatica* powder; (5) normal diet + 0.03% (v/v) H₂O₂ + 5.0% (w/w) *C. asiatica* powder and (6) normal diet + 0.03% (v/v) H₂O₂ + 0.3% (w/w) α -tocopherol. Initially, all rats were acclimatized (fed with commercial diet) for 2 weeks. Weight gain and amount of feed taken were recorded every week. Amount of feed taken by each rat was calculated according to the average of feed consumed by rats in the same group.

The concentrations of 0.3% (w/w) *C. asiatica* extract, 1.5% (w/w) and 5.0% (w/w) *C. asiatica* powder were chosen because of the following reasons. According to Abdul-Hamid et al. (2002), the antioxidant activity of *C. asiatica* extract at a concentration of 3,000 ppm and above was not significantly different ($p > 0.05$) from the antioxidant activity of α -tocopherol at the same concentration. Therefore, 0.3% (w/w) of *C. asiatica* extract was selected to be used in this study.

Previous study done by Abdul-Hamid et al. (2002) revealed that extracting *C. asiatica* powder with a polar solvent (water) gave an average of 20% yield of *C. asiatica* extract. In order to obtain the 20% yield of herbal extract, 100 g of herbal powder was required for the extraction. In view of this, for 0.3% (w/w) herbal extract treatment diet, an amount of 1.5% (w/w) herbal powder was required to achieve such a yield of extract. Therefore, 1.5% (w/w) *C. asiatica* powder (1.5 g *C. asiatica* powder/100 g commercial rat's feed) treatment diet was used in this study as a comparison against 0.3% w/w *C. asiatica* extract treatment diet.

On the other hand, a high percentage of *C. asiatica* powder (5.0%) was used in this study to determine the effect of other components in *C. asiatica* such as fibre, chlorophyll and carbohydrate on H₂O₂-treated rats. The percentage of 0.3% (w/w) α -tocopherol used was parallel with 0.3% (w/w) *C. asiatica* extract which exhibited no significant difference ($p > 0.05$) in antioxidant activity against *C. asiatica* extract at 3,000 ppm as stated earlier.

A trial level of 0.03% (v/v) H₂O₂ in inducing oxidative stress in Sprague-Dawley rats was in accordance to Umemura et al. (1996). They examined oxidative stress in the livers of B6C3F1 mice fed with pentachlorophenol in their diet at doses of 0.03% to 0.12%.

Blood sampling and preparation of red blood cells (RBC)

The first blood samples were taken at the end of the second week of acclimatization with the commercial feed. This was for analysis of basal data before treatment commenced. Throughout the study, blood samples were taken at week 0 and 6 of the dietary treatments for preparation of red blood cells and determination of lipid peroxidation.

About 2–3 ml of rat's blood was collected by cardiac puncture and drawn into a vacutainer tube containing ethylenediaminetetraacetic acid (EDTA) and centrifuged at 3,000 rpm for 15 min at 4 °C. The plasma was removed and the sediment containing erythrocytes was suspended in isotonic saline (sodium chloride-NaCl) and re-centrifuged. This process was repeated twice. A stock haemolysate was prepared by adding 4 parts (by volume) of ice-cold distilled water to the sediment (RBC) and mixed thoroughly. This haemolysate was used for the estimation of lipid peroxidation (malonaldehyde).

Adult male Sprague-Dawley rats (approximately 3 months old, weighing in the range of 300–500 g) were used in this study due to the big volume of blood samples required in order to run the analysis of blood parameters stated. This is in accordance to Waynforth and Flecknell (1994), which revealed that if the rats are to be allowed to recover from the cardiac puncture procedure, the volume of blood removed should be limited to 10–15% of circulating volume which is approximately 7–10 ml blood/kg. Since the weight of rats used in this study was 300 g and above, the maximum blood withdrawn (2–3 ml) was enough for the blood parameters determination.

Determination of lipid peroxidation

Lipid peroxidation of RBC was measured by thiobarbituric acid reaction by the method of Buege and Aust (1978). RBC (0.5 ml) was mixed with 5 μ l of 10 mM EDTA and 1 ml

(TBA-TCA-HCl) solution and placed in boiling water bath for 15 min. After cooling, the flocculent precipitate was removed by centrifugation at 1,000 g for 10 min. The absorbance of red colour of TBA-malonaldehyde complex was then measured at 532 nm against a blank that contained all the reagents except the lipid. MDA formed from the breakdown of polyunsaturated fatty acids was calculated using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Histopathology observations

The rats were sacrificed at the end of the feed trials and samples of heart, liver and kidney were collected for histopathological examination. Histopathology samples were prepared according to the method of Luna (1968). Organ samples of heart, liver and kidney of the rats were fixed in 10% buffer formalin for at least 48 h for histopathological examinations. The tissues (0.5 cm) were trimmed and the blocks were subsequently dehydrated in series of alcohol, cleared with xylene and embedded in paraffin wax using an Automatic Tissue Processor, Histokinette 2000 (Reichert-Jung, Cambridge Instruments Inc., New York, USA). Tissues were then sectioned at about 5 μm on a microtome (Jung Multicut 2045, Cambridge Instruments Inc., USA) and mounted on glass slides, followed by dewaxing and stained with haematoxylin and eosin. Tissues were carefully examined under a microscope and the histological changes for each organ were recorded for each group.

Statistical analysis

Results obtained were expressed as mean \pm standard deviation and statistical analysis was done according to the SAS (1990) User's Guides. Data were analysed using one-way analysis of variance (ANOVA) and general linear model (GLM). Duncan multiple range test (DMRT) was used to examine differences between groups and among groups means. A p value <0.05 was considered statistically significant.

Results and discussion

Dietary intake, body weight and organ weight of rats

In general, results showed that daily food intake of the different groups of rats were not statistically different ($p >0.05$) throughout the study (Figure 4). There were no significant ($p >0.05$) differences in weight gain between groups for the whole of 5 weeks treatments (Figure 5). However, at week 6, the average body weights of the rats treated with 0.3% (w/w) *C. asiatica* extract and 1.5% (w/w) *C. asiatica* powder were statistically ($p <0.05$) lower than that fed with 5.0% (w/w) *C. asiatica* powder but not significantly ($p >0.05$) different compared to normal and H_2O_2 -treated rats.

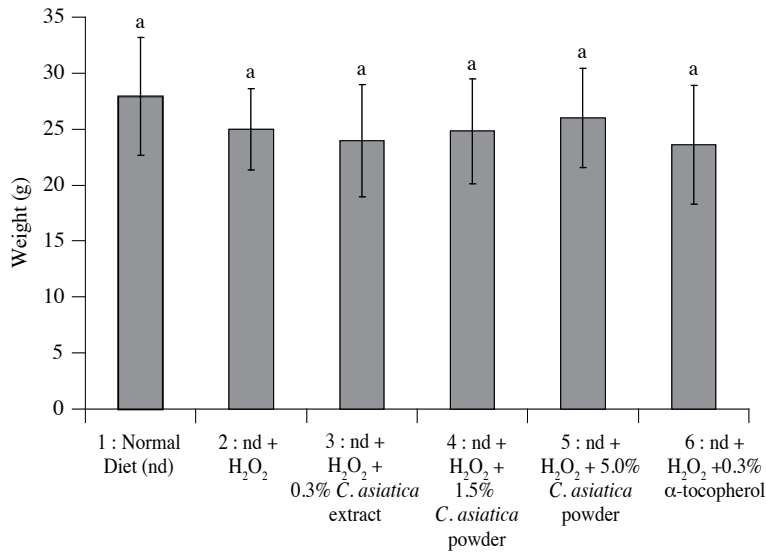
The reduction in body weight of *C. asiatica* extract supplemented rats was similar to the findings observed by Jouad et al. (2003) and Coelho et al. (2004) which demonstrated significant reduction in body weight of rats treated with *Spergularia purpurea* and *Baccharis genistelloides* aqueous extracts respectively. One possible mechanism contributing towards reduction in body weight of rats supplemented with *C. asiatica* extract is the enhancing breaking down of fats stimulated by catechin compound which was found in high levels in *C. asiatica* (Spurlock et al.1996; Zainol et al. 2003).

Centella asiatica consumed by the rats did not interfere with the diet digestion and had no toxic effect on the rats since no significant ($p >0.05$) weight gain was observed compared to normal and H_2O_2 -treated rats. This finding was found to be similar to Hori et al. (1994) and Matsushashi et al. (1997). The mean weights of heart, liver and kidney of the rats between groups were not affected by *C. asiatica* extract and powder; α -tocopherol or by H_2O_2 since no significant ($p >0.05$) differences were noted in the weight of these organs (Figure 6).

Lipid peroxidation

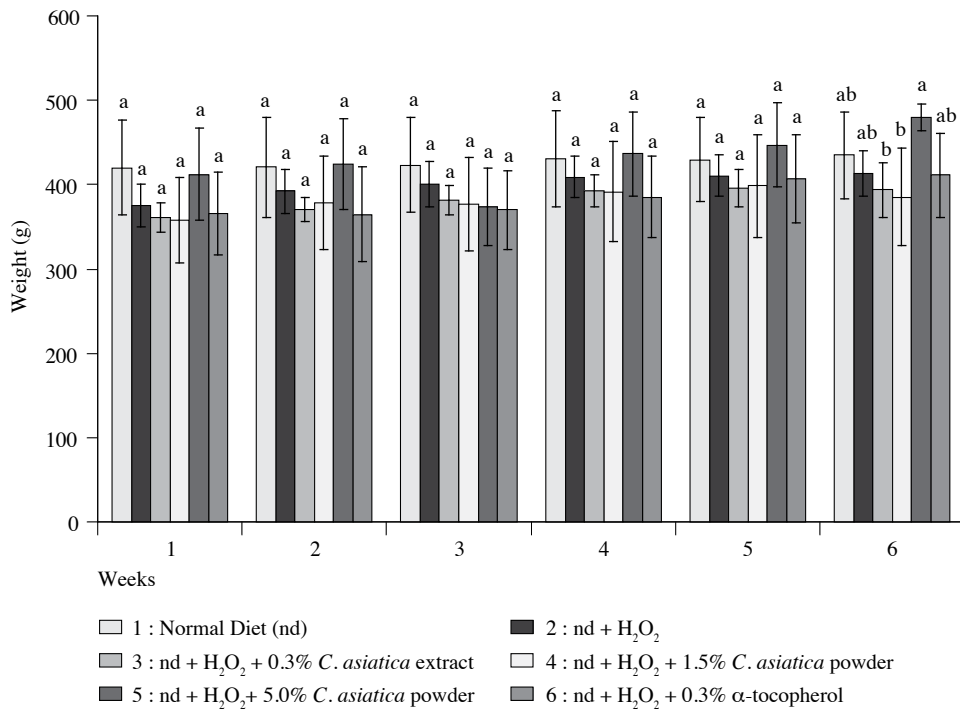
Like other biological membranes, red blood cell membrane is also prone to lipid

Effect of *Centella asiatica* on Sprague-Dawley rats



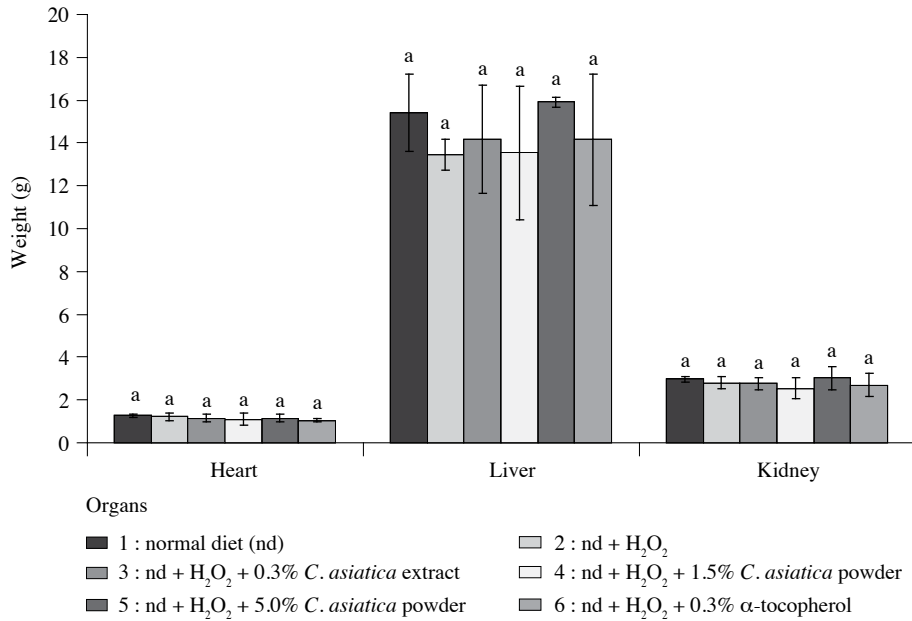
Values with same letter (a) are not significantly different ($p > 0.05$) between group treatments

Figure 4. Dietary intake (g/day/rat) of *Sprague-Dawley* rats fed with different diets



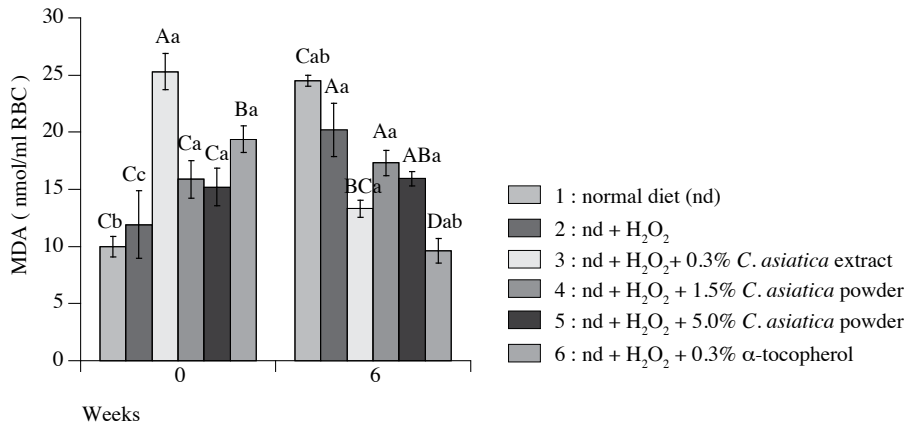
Values with different letters (a–b) are significantly different ($p < 0.05$) among group treatments within a week

Figure 5. Mean body weight of *Sprague-Dawley* rats fed with different diets for 6 weeks



Values with same letter (a) are not significantly different ($p > 0.05$) among group treatments within an organ

Figure 6. Mean organs weight of Sprague-Dawley rats fed with different diets



A–D: Values with different letters are significantly different ($p < 0.05$) among different groups treatments within a week

a–c: Values with different letters are significantly different ($p < 0.05$) among a group at different weeks of treatments

Figure 7. Malonaldehyde concentration of Sprague-Dawley rats before (week 0) and after treatment (week 6) with different diets

peroxidation under oxidative stress involving cleavage of polyunsaturated fatty acids at their double bonds leading to formation of MDA (Halder and Bhaduri 1998). Lipid peroxides produced from unsaturated fatty acid by radicals are histotoxic, thus able to increase production of free radicals in a manner of chain reactions (Fong et al. 1973; Weiss et al. 1977).

Levels of lipid peroxidation in all groups are shown in Figure 7. MDA levels of normal and H₂O₂-treated rats were significantly ($p < 0.05$) increased at week 6 compared to week 0, while α -tocopherol supplemented rats demonstrated significantly ($p < 0.05$) lower MDA level among different treatments. It is interesting to note that at the end of the study, rats supplemented with both *C. asiatica* extract and powder (groups 3, 4 and 5) significantly ($p < 0.05$) demonstrated lower MDA production, as compared to normal and H₂O₂-treated rats. These observations thus suggest that dietary supplementation of *C. asiatica* and α -tocopherol is effective in lowering MDA production and therefore reduced lipid peroxidation in rats.

Histological observations

Histological examination revealed mild to moderate deposition of adipose tissues on the capsular surfaces of the liver, kidney and pericardium of the rats given the normal diet. These rats were also found to have mild degeneration of hepatocytes and renal tubules with mild fatty degeneration of the hepatocytes and few hepatocytes cells were seen to undergo apoptosis or necrosis. In addition, congestion of the kidney, liver and heart were also observed.

Similar findings were seen in H₂O₂-treated rats except that deposition of fat tissues on the capsular surfaces of liver, kidney and pericardium were less consistent than that of normal rats. Histological examinations of the organs of rats given the different treatments were similar to that found in H₂O₂-treated rats. Based on

these findings, in general, there were no significant changes observed in the different group treatments.

Conclusion

The study indicated that although 0.03% (v/v) H₂O₂ given to the experimental rats was probably not sufficient in inducing oxidative stress, MDA level was found to be significantly ($p < 0.05$) higher in normal rats compared to *C. asiatica* and α -tocopherol supplemented rats. Thus, *C. asiatica* powder and extract significantly ($p < 0.05$) decreased the MDA level and therefore lowered lipid peroxidation in the experimental rats. On the other hand, 0.3% *C. asiatica* extract and 1.5% *C. asiatica* powder significantly ($p < 0.05$) reduced body weight of the rats at the end of the treatment (week 6) compared to 5.0% *C. asiatica* supplemented rats. No significant differences were observed in dietary intake and histology examination of the organs in all rats.

References

- Abdul-Hamid, A., Md.-Shah, Z., Muse, R. and Mohamed, S. (2002). Characterisation of antioxidant activities of various extracts of *Centella asiatica* (L) Urban. *Food Chemistry* 77: 465–469
- Ames, B.N., Shigenaga, M.K. and Hagen, T.M. (1993). Oxidants, anti-oxidants and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences USA* 90: 7915–7922
- Aruoma, O.I. (1996). Assessment of potential prooxidant and antioxidant actions. *JAOCs* 73(12): 1617–1625
- Buege, A. and Aust, S.D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology* 52: 302–310
- Burkill, I.H. (1966). *A dictionary of the economic products of the Malay Peninsula* (Vol.1 and 2). Min. Agric. & Coop. Govt. of Malaysia and Singapore
- Clement, M.V., Ponton, A. and Pervaiz, S. (1998). Apoptosis induced by hydrogen peroxide is mediated by decreased superoxide anion concentration and reduction of intracellular milieu. *FEBS Letters* 440(1–2): 13–18
- Coelho, M.G.P., Reis, P.A., Gava, V.B., Marques, P.R., Gayer, C.R., Laranja, G.A.T., Felzenswalb, I. and Sabino, K.C.C.

- (2004). Anti-arthritis effect and subacute toxicological evaluation of *Baccharis genistelloides* aqueous extract. *Toxicology Letters* 154: 69–80
- Fallarero, A., Loikkanen, J. J., Mannisto, P.T., Castaneda, O. and Vidal, A. (2003). Effect of aqueous extracts of *Halimeda incrassata* (Ellis) Lamouroux and *Bryothamnion triquetrum* (S.G.Gmelim) Howe on hydrogen peroxide and methyl mercury-induced oxidative stress in GT1-7 mouse hypothalamic immortalized cells. *Phytomedicine* 10: 39–47
- Farber, J.L., Kyle, M.E. and Coleman, J.B. (1990). Mechanisms of cell injury by activated oxygen species. *Lab Invest* 62: 670–679
- Fong, K.L., McCay, P.B., Poyer, J.L., Keele, B.B. and Misra, H. (1973). Evidence that peroxidation of lysosomal membranes is initiated by hydroxyl free radicals produced during flavin enzyme activity. *J. Biol. Chem.* 248: 7792–7797
- Goh, S.H., Chuah, C.H., Mok, J.S.L. and Soepadmo, E. (1995). *Malaysian medicinal plants for the treatment of cardiovascular diseases*. Kuala Lumpur: Academe Art and Printing services Sdn. Bhd.
- Gunther, B. and Wagner, H. (1996). Quantitative determination of triterpenes in extracts and phytopreparations of *Centella asiatica* (L.) Urban. *Phytomedicine* 3: 59–65
- Halder, J. and Bhaduri, A.N. (1998). Protective role of black tea against oxidative damage of human red blood cells. *Biochemical and Biophysical Research Communications* 244: 903–907
- Halliwel, B. and Aruoma, O.I. (1991). DNA damage by oxygen-derived species : its mechanism and measurement in mammalian systems. *FEBS Letter* 281: 9–19
- Halliwel, B. and Gutteridge, J.M.C. (1999). *Free radicals in biology and medicine*, 3rd Edition. Oxford, UK: Clarendon Press
- Hampton, M. and Orrenius, S. (1997). Dual regulation of caspase activity by hydrogen peroxide: implications for apoptosis. *FEBS Letters* 414(3): 552–556
- Hori, K., Ishibashi, G. and Okita, T. (1994). Hypocholesterolemic effect of blue-green alga, ishikurage (*Nostoc commune*) in rats fed atherogenic diet. *Plant Foods for Human Nutrition* 45: 63–70
- Hsieh, T.J., Liu, T.Z., Chia, Y.C., Chern, C.L., Lu, F.J., Chuang, M.C., Mau, S.Y., Chen, S.H., Syu, Y.H. and Chen, C.H. (2004). Protective effect of methyl gallate from *Toona sinensis* (Meliaceae) against hydrogen peroxide-induced oxidative stress and DNA damage in MDCK cells. *Food and Chemical Toxicology* 42: 843–850
- Jaganath, I.B. and Teik, N.L. (2000). *Centella asiatica*. In: *Herbs – The green pharmacy of Malaysia*, p. 21–25. Kuala Lumpur: Vinpress
- Jayaweera, D.M. (1982). *Medicinal plants (indigenous and exotic) used in Ceylon*, Part 5, 134 p. Colombo: The National Science Council of Sri Lanka
- Jouad, H., Lemhadri, A., Maghrani, M., Zeggwagh, N.A. and Eddouks, M. (2003). Cholesterol-lowering activity of the aqueous extract of *Spergularia purpurea* in normal and recent-onset diabetic rats. *Journal of Ethnopharmacology* 87: 43–49
- Kartnig, T. (1988). Clinical applications of *Centella asiatica* (L.) Urb. in herbs, spices and medicinal plants. *Recent advances in botany, horticulture and pharmacology* 3: 145–173
- Luna, L.G. (1968). *Manual of histologic staining methods of the Armed Forces Institute of Pathology*, 3rd Edition. New York, USA: The Balakiston Division, McGraw-Hill Book Company
- Luo, S. and Jin, H. (1981). Isolation and identification of madecassoside in *Centella asiatica*. *Zhongcaoyao* 12: 5–6
- Matsuhashi, T., Karbowski, M., Liu, X., Usukura, J., Wozniak, M. and Wakabayashi, T. (1997). Complete suppression of ethanol-induced formation of megamitochondria by 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (4-OH-TEMPO). *Free Radical Biology and Medicine* 24: 139–147
- Mohamad-Khairi, M. Z., (2004). Determination of flavonoids in *Centella asiatica* (L.) Urban and their utilization in herbal noodle. MSc Thesis, University Putra Malaysia
- Rimpler, M.M., Rauen, U., Schmidt, T., Moroy, T. and De-Groot, H. (1999). Protection against hydrogen peroxide cytotoxicity in Rat-1 fibroblasts provided by the oncoprotein bcl-2: maintenance of calcium homeostasis is secondary to the effect of bcl-2 on cellular glutathione. *Biochemistry Journal* 340: 291–297
- Sahu, N.P., Roy, S.K. and Mahato, S.B. (1989). Spectroscopic determination of structures of triterpenoid trisaccharides from *Centella asiatica*. *Phytochemistry* 28: 2852–2854
- Singh, B. and Rastogi, R.P. (1968). Chemical examination of *Centella asiatica*. III. Constitution of brahmic acid. *Phytochemistry* 7: 1385–1393
- Spurlock, M.E., Hahn, K.J. and Miner, J.L. (1996). Regulation of adipin and body composition

- in the monosodium glutamate (MSG)-treated mouse. *Physiology & Behavior* 60:1217–1221
- Umemura, T., Sai-Kato, K., Takagi, A., Hasegawa, R. and Kurokawa, Y. (1996). Oxidative DNA damage and cell proliferation in the livers of B6C3F1 mice exposed to pentachlorophenol in their diet. *Fundamental and Applied Toxicology* 30: 285–289
- Waynforth, H.B. and Flecknell, P.A. (1994). *Experimental and surgical technique in the rat*, 2nd Edition. London: Academic Press
- Weiss, S.J., King, G.W. and LaBuglio, A.F. (1977). Evidence for hydroxyl radical generation by human monocytes. *J. Clin. Invest* 60: 370–373
- Zainol, M.K.; Abdul-Hamid, A.; Yusof, S. and Muse, R. (2003). Antioxidative activity and total phenolic compounds of leaf, roots and petioles of four accessions of *Centella asiatica* (L.) Urban. *Food Chemistry* 81: 575–581

Abstrak

Satu kajian telah dijalankan untuk mengkaji kesan ekstrak dan serbuk pegaga (*Centella asiatica*) terhadap pengambilan diet, berat badan, berat organ dan pengoksidaan lemak darah pada tikus jantan *Sprague-Dawley* yang telah dirawat dengan hidrogen peroksida (H_2O_2). Tikus uji kaji telah diberi 0.3% (berat/berat) ekstrak *C. asiatica*, 1.5% (berat/berat) dan 5.0% (berat/berat) serbuk *C. asiatica* dan 0.3% (berat/berat) α -tokoferol selama 6 minggu. Stres oksidatif pada tikus ini telah dirangsang dengan memberikan 0.03% (isi padu/isi padu) H_2O_2 di dalam air minuman. Jumlah pengambilan diet, berat organ dan badan serta aras malonaldehid (MDA) sepanjang tempoh kajian telah dipantau. Ujian histopatologi organ terpilih telah dilakukan pada akhir kajian. Aras MDA tikus normal lebih tinggi berbanding dengan tikus uji kaji yang lain. Penambahan *C. asiatica* (ekstrak dan serbuk) dan α -tokoferol di dalam diet telah mengurangkan pengoksidaan lemak dengan signifikan ($p < 0.05$) pada tikus uji kaji. Walau bagaimanapun, tiada perubahan yang signifikan dapat dilihat pada pengambilan diet dan pemerhatian histopatologi organ tikus.