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Food Chemistry 100 (2007) 535–541
Food Chemistry
www.elsevier.com/locate/foodchem

Protective effect of *Centella asiatica* extract and powder on oxidative stress in rats

Mahanom Hussin [a], Azizah Abdul-Hamid [a,*], Suhaila Mohamad [a], Nazamid Saari [a], Maznah Ismail [b], Mohd. Hair Bejo [c]

[a] *Department of Food Science, Faculty of Food Science and Biotechnology, University Putra Malaysia, 43400 UPM UPM Serdang, Selangor, Malaysia*
[b] *Interim Institute, Pharmaceutical and Nutraceutical Biotechnology, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[c] *Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

Received 7 June 2004; received in revised form 11 October 2005; accepted 11 October 2005

Abstract

The effect of *Centella asiatica* extract and powder in reducing oxidative stress in *SpraqueDawley* rats was evaluated. Lipid peroxidation was monitored by measuring malonaldehyde (MDA) level in blood. Activities of free radical-scavenging enzymes (superoxide dismutase and catalase) were determined using H_2O_2 decomposition and nitrobluetetrazolium reduction, respectively. Results showed that administration of H_2O_2 (0.1%) in drinking water of the rats, for 25 weeks, increased the malonaldehyde levels in erythrocytes of all the rats. However, rats receiving *C. asiatica* extract, powder and α-tocopherol had lower MDA levels than did the other rats, which indicates, decrease lipid peroxidation in these rats. Increase in catalase activity of the rats appears to be a response to H_2O_2 accumulation. The decrease in the activity of superoxide dismutase in *C. asiatica*- and α-tocopherol supplemented rats suggested a lower requirement for the enzyme and this indicates the protective effect of the plant in combating oxidative stress undergone by the rats. Results revealed that *C. asiatica* extract and powder may ameliorate H_2O_2-induced oxidative stress by decreasing lipid peroxidation via alteration of the antioxidant defence system of the rats.

Keywords: *Centella asiatica*; Oxidative stress; Malonaldehyde; Antioxidant enzymes

1. Introduction

Popularity of *Centella asiatica* or pegaga as one of the local medicinal plants, is mostly due to its reputation as a wound healing agent and brain stimulant (Bonte, Dumas, Chaudagne, & Meybecle, 1994; Kathy, 2000). *C. asiatica,* which grows wild in both tropical and sub-tropical countries, is closely related to the species Hydrocotyle and produces characteristic essential oil (Yoshinori, Reiko, & Tsunematsu, 1982) and various types of flavonoid (Nakoki & Morita, 1960). The herb is said to have a direct effect in lowering blood pressure and is often referred to as a rejuvenating medicament in the Ayurvedic Pharmacopoeia (Jayaweera, 1982). In addition, it is also believed to be able to purify blood, cure indigestion and nervousness, treat skin disorders, and as a diuretic, and antihypertensive agent, and as a remedy against asthma, leprosy, anaemia and inflammations (Jayatilake & MacLeod, 1987; Ramaswamy, Periyasamy, & Basu, 1970). Numerous clinical reports verify the ulcer-preventive (Huriez, 1972) and antidepressive-sedative (Sakina & Dandiya, 1990) effects of *C. asiatica* preparations, as well as their ability to improve venous insufficiency (Monteverde, Occhipinti, Rossi, & Vellata, 1987; Kartnig, 1988).

Hydrogen peroxide (H_2O_2) has been shown to induce oxidative stress in both human and animal models, leading to the generation of potent reactive oxygen species (ROS), such as hydroxyl radical ($OH^{\cdot}$). Oxidative stress results

* Corresponding author. Tel.: +60 3 89468387/89486101; fax: +60 3 89423552.
E-mail address: azizah@fsb.upm.edu.my (A. Abdul-Hamid).

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doi:10.1016/j.foodchem.2005.10.022

when generation of reactive oxygen and/or nitrogen species and activity of the antioxidant defences are unbalanced. The increase in ROS could be due to their excessive production and/or decreased destruction. Cells exposed to severe oxidative stress may suffer degeneration of DNA, membrane lipids and protein and enzymes, leading to various pathological conditions. Ward, Blakely, and Joner (1985) have suggested that the cytotoxicity of H_2O_2 is associated with local multiplication of damaged sites on DNA. Thus, it is imperative that H_2O_2 levels in tissues be minimized. Such oxidative damage is known as one of the mechanisms leading to chronic diseases, such as atherosclerosis, aging, cancer and rheumatoid arthritis (Aruoma, 1998; Halliwell & Gutteridge, 1989). Antioxidant enzymes, in particular superoxide dismutase, catalase and glutathione peroxidase, represent cell defence mechanisms for preventing oxidative damage.

Plants are known to produce various antioxidant compounds to interact with ROS in order to survive (Lu & Foo, 1995). Therapeutic properties of some of these plant extracts used in traditional medicine have been linked to their antioxidative activities. *C. asiatica* has been reported to contain numerous caffeic acid derivatives and flavonols and in particular, quercetin and kaempferol (Castellani, Marai, & Vacchi, 1981), catechin, rutin and naringin (Zainol, Abdul-Hamid, Yusof, & Muse, 2003), some of which have been shown to be potent antioxidants.

In this study, the efficacy of *C. asiatica* extract and powder in preventing lipid peroxidation was studied by monitoring its effect on antioxidant enzymes in oxidative stress induced male *Sprague dawley* rats.

2. Materials and methods

2.1. Raw materials

C. asiatica extract and powder were provided by the Forest Research Institute of Malaysia (FRIM).

2.2. Chemicals and reagents

Thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), nitrobluetetrazolium (NBT), Triton X-100, riboflavin, bovine serum albumin and folin Ciocalteul reagent were purchased from Sigma Chemical Co. St. Louis, Missouri. All reagents were of analytical grade.

2.3. Animals and diets

Seventy adult *S. dawley* rats, approximately 3 months old, weighing in the range of 300–487 g, were selected for the study. All the rats were healthy and not infected with virus or bacteria. The rats were housed for 25 weeks in separate cages (7 rats per cage) 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, Table 1) with free access to basal, treatment diet and water ad libitum. The rats were randomly divided into five groups of 14 animals and treated for 25 weeks as follows: Group 1 was given only normal diet; Group 2 was given normal diet and 0.1% H_2O_2 in drinking water; Group 3 was given normal diet, 0.1% H_2O_2 and 0.3% *C. asiatica* extract; Group 4 was given normal diet, 0.1% H_2O_2 and 5.0% *C. asiatica* powder; and Group 5 received normal diet, 0.1% H_2O_2 and 0.3% α-tocopherol. Initially, all the rats were fed commercial diet for 2 weeks. Blood samples were then taken at the end of the second week. Blood was collected by cardiac puncture and drawn into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) for erythrocyte preparations or drawn into plain vacutainers for serum collection. Throughout the study, blood samples were taken at specified weeks for 6 months and selected parameters were then determined. Food consumption, general conditions and any other symptoms were observed daily and weight gains were recorded weekly. At the end of the experiment, all the rats were sacrificed and organ weights, namely heart, liver and kidney, were determined.

Table 1
Composition of commercial diet

Ingredients	Percent, % (g/100 g diet)
Crude protein	22
Crude fibre	5
Crude fat	3
Moisture	13
Ash	8
Nitrogen free extract	49
Vitamins and minerals	Enriched

2.4. Preparation of red blood cells and serum

Rat's blood was drawn into vacutainer tubes containing EDTA and centrifuged at 3000 rev./min for 15 min. at 4 ± 1 °C. Plasma was then removed and the sediment containing erythrocytes was suspended in saline (NaCl) and re-centrifuged. This process was repeated twice. Stock haemolysate was prepared by adding four parts of ice-cold distilled water to the sediment (RBC). The haemolysate was later used for the determination of lipid peroxidation (malonaldehyde) and catalase activity. In serum preparation, rat's blood was collected in plain vacutainer tubes and allowed to clot for 60 min (37 °C), followed by centrifugation at 3000 rev./min for 10 min (37 °C). The supernatant was then used for measurement of superoxide dismutase activity.

2.5. Lipid peroxidation

Lipid peroxidation of red blood cells was measured using the thiobarbituric acid test, employing the method of Buege and Aust (1978). Red blood cells (0.5 ml) were mixed with 5 μl of 10 mM EDTA and 1 ml (TBA-TCA-HCl) solution and placed in a boiling water bath for

15 min. After cooling, the flocculent precipitate was removed by centrifugation at 1000*g* for 10 min. The absorbance of red TBA-malonaldehyde complex was then measured at 532 nm. Malonaldehyde formed from the degradation of polyunsaturated fatty acids, was then calculated using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

2.6. Free radicals scavenging enzymes

2.6.1. Superoxide dismutase (SOD)

Superoxide dismutase activity was determined according to the method of Beyer and Fridovich (1987). Enzyme activity was measured by mixing phosphate buffer, pH 7.8, containing 0.1 mM EDTA, L-methionine, Nitro blue tetrazolium and Triton X-100. Sample (20 μl) was then added to the mixture, followed by addition of riboflavin (10 μl). The tubes were then illuminated for 7 min in an aluminium foil-lined box containing two 20 W fluorescent lamps. A control tube, in which sample was replaced by buffer, was also run and the absorbance measured at 560 nm. One unit of superoxide dismutase activity is defined as the amount of sample required to decrease 50% SOD-inhibitable nitro blue tetrazolium.

2.6.2. Catalase

Catalase activity was measured utilising the method of Aebi (1974). Sample (2.0 ml haemolysate) was added to a cuvette containing 1.0 ml (50 mM) phosphate buffer (pH 7.0). The reaction was started by adding 1.0 ml of freshly prepared 30 mM hydrogen peroxide (H_2O_2) to 2.0 ml sample (haemolysate) in a cuvette. The decomposition of H_2O_2 was determined directly by the decrease in extinction at 240 nm and the difference in extinction (ΔE_{240}) per unit time was used as a measure of catalase activity. One unit of activity is defined as sample that will decompose 1.0 μmole of H_2O_2 per min at pH 7.0 at 25 °C, while the H_2O_2 concentration falls from 10.3 to 9.2 mM.

2.7. Statistical analysis

Results obtained were expressed as means ± SEM and statistical analysis was done according to the SAS User's Guides (1990). Data were analyzed using one-way, and two-way analysis of variance and a general linear model (GLM). Duncan's multiple range test (DMRT) was used to examine differences between groups and among group means. A p value <0.05 was considered statistically significant.

3. Results and discussion

3.1. General

Currently, there is considerable interest in research involving free radical mediated damage to biological systems, which can lead to dysfunction of various tissue components. Several lines of evidence suggest that free radical scavengers, especially certain antioxidants, may offer a preventive measure or cure for a large number of human conditions (Vendemiale, Grattagliano, & Altomare, 1999). However, there is lack of consensus in supporting the use of natural plant products, in particular, herbs as oxidation interventions for scavenging free radicals. Thus, a study of the most commonly used medicinal plant, *C. asiatica*, in combating oxidative stress appeared to be of interest.

3.2. Dietary intake, body weight and organ weight of rats

Significant ($p < 0.05$) changes were observed in food consumption, weight gain and liver weights of *C. asiatica*- and α-tocopherol-fed rats compared to that of control rats during the study (Figs. 1–3). Fig. 1 shows that food consumptions of both *C. asiatica*- and α-tocopherol-fed rats were significantly lower than that of control. However, at the end of the study, heart and kidney weights did not show any significant differences in any groups. This suggested

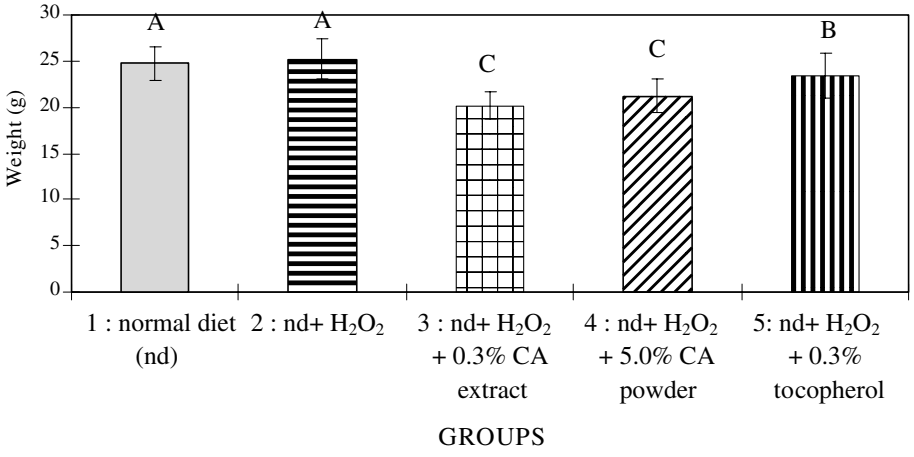

Fig. 1. Dietary intake (g per day per rat) of *S. dawley* rats throughout 25 weeks of treatments. (A–C): Values with different letters are significantly different ($p < 0.05$) between group treatments.

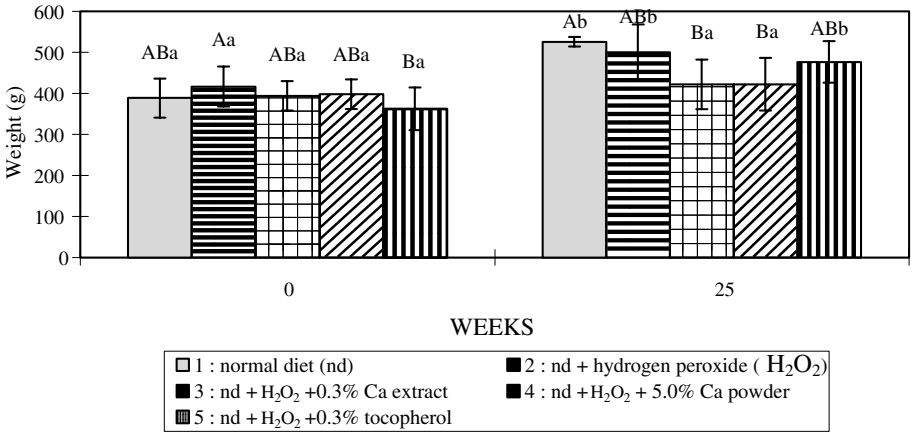

Fig. 2. Mean body weight of experimental rats before (week 0) and after treatments (week 25). (A–C): Values with different letters are significantly different ($p < 0.05$) among different groups treatments within a week. (a–b): Values with different letters are significantly different ($p < 0.05$) among a group at different weeks of treatments.

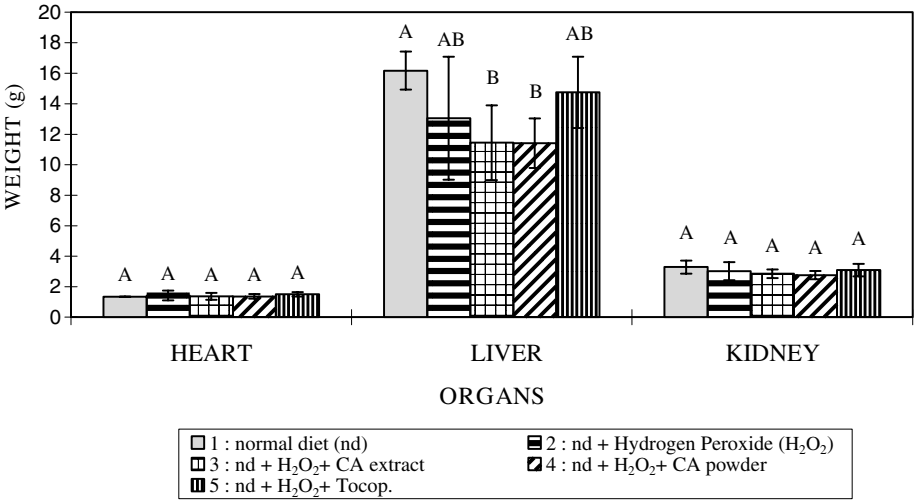

Fig. 3. Mean organs weight of *S. dawley* rats fed with normal and treatment diets. (A–B): Values with different letters are significantly different ($p < 0.05$) among different group treatments within an organ.

that addition of *C. asiatica* to the diets may have resulted in reduced appetite of the rats and it therefore significantly lowers the food intake of the rats compared to those fed a normal diet. This is reflected by the significant reduction in body weights observed in *C. asiatica*-fed rats. This is true for both *C. asiatica* extract- and powder-fed rats where their body weights were found to be maintained throughout the study. The lowered body weights achieved by *C. asiatica*-fed rats in comparison to that of control rats, may also be a result of enhanced breaking down of fats, stimulated by catechin content of pegaga (Spurlock, Hahn, & Miner, 1996). Zainol et al. (2003) have reported that *C. asiatica* contained high levels of catechin. Similar results were not seen in α-tocopherol-supplemented rats.

3.3. Effect of C. asiatica on lipid peroxidation

Lipid peroxidation is an auto-catalytic, free-radical-mediated, destructive process, whereby polyunsaturated fatty acids in cell membranes undergo degradation to form lipid hydroperoxides (Sevanian, 1985; Slater, 1984). These latter compounds then decompose to form a wide variety of products, including low molecular mass hydrocarbons, hydroxy aldehydes, fatty acids, ketones, alkenals and alkanals, in particular malonaldehyde (MDA) (Zeyuan, Bingyin, Xiaolin, Jinming, & Yifeng, 1998), Thus, reduction of MDA production would indicate inhibition of lipid peroxidation.

Fig. 4 reveals that malonaldehyde contents in the red blood cell (RBC) of the rats significantly increased in rats receiving normal diet. However, MDA levels of H_2O_2-treated rats were seen to increase only to a limited extent; the levels were still significantly lower than that of normal diet at week 25. Similar findings were seen in both *C. asiatica* extract- and powder-fed groups and the α-tocopherol-supplemented group. MDA level in these rats were found to be significantly lower than in normal rats after treatment ended at week 25, where, interestingly, the *C.*

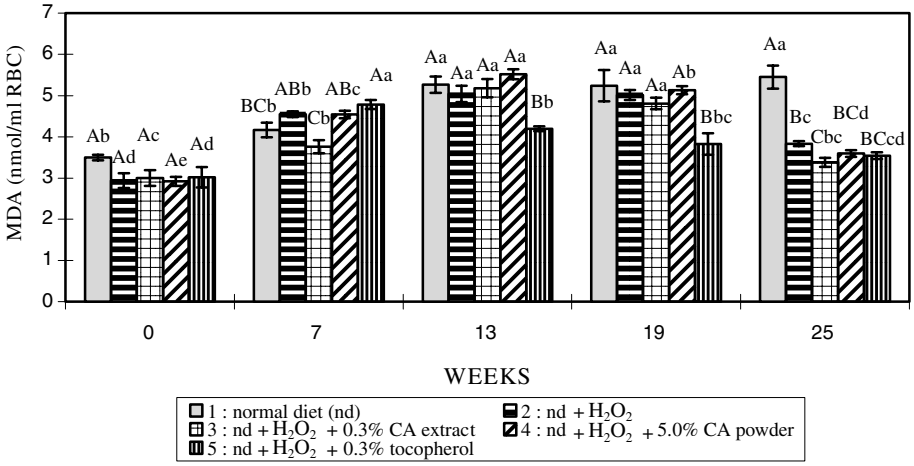

Fig. 4. Malonaldehyde production of *S. dawley* rats before and after treatments. (A–C): Values with different letters are significantly different ($p < 0.05$) among different group treatments within a week. (a–d): Values with different letters are significantly different ($p < 0.05$) among a group at different weeks of treatments.

asiatica extract-fed group had the lowest MDA, which was even lower than that of the rats fed tocopherol, although they were not significantly different. Malonaldehyde content was evaluated as an endpoint, indicative of the extent of lipid peroxidation, since MDA is known to be one of the most abundant aldehydes formed as a byproduct of lipid peroxidation (Gurer, Ozgunes, Neal, Spitz, & Ercal, 1998).

The significant increase of MDA level in the RBC of normal diet and H_2O_2-treated rats indicated the possibility of increased radical production, and higher rate of lipid peroxidation in these rats. However, the MDA increase, experienced by normal rats, may also be associated with aging (Inal, Kanbak, & Sunal, 2001). Rodriguez and Ruiz (1992) indicated that plasma MDA levels were increased with age in healthy subjects and Nohl (1991) reported accumulation of lipid peroxidation products during aging.

Previous study in this laboratory showed that different accessions of *C. asiatica* exhibited a high antioxidative activity that was comparable to that exhibited by both α-tocopherol and BHT (Zainol et al., 2003). This is especially true for the root and leaf of the same, which the authors attributed to the phenolic compound present in the plant. Phenolic compounds have been reported to possess powerful antioxidative properties (Maxwell, Cruikshank, & Thorpe, 1994; Serafini, Ghiselli, & Ferro-Luzzi, 1994) and are regarded as a class of semiessential food components regarded as potentially important for the prevention of chronic diseases in man (Zeyuan et al., 1998). The reduction of MDA in *C. asiatica*-fed groups at the end of the study might represent antioxidative augmentation and prove that *C. asiatica* has excellent antioxidation activity, as previously measured. The results of the study suggested that *C. asiatica* and α-tocopherol pretreatment may decrease lipid peroxidation and hence protect the rats undergoing H_2O_2-treatment from possible oxidative damage.

3.4. Effect of C. asiatica on the activities of free radical-scavenging enzymes

Biological effects of ROS are controlled in vivo by a wide spectrum of enzymatic and non-enzymatic defence mechanisms, in particular superoxide dismutase, which catalyzes dismutation of superoxide anions to hydrogen peroxide and catalase, which then converts H_2O_2 into molecular oxygen and water (Inal et al., 2001). Their roles as protective enzymes are well known and have been investigated extensively both in vivo and in model systems (Wheeler, Salzman, Elsayed, Omaye, & Korte, 1990). Superoxide dismutase represents the front line of defence against oxidative damage (Hassan & Schellhorn, 1988) and provides defence against toxicity of dioxygen (Fridovich, 1989). Catalase, on the other hand, has been suggested to provide an important pathway for H_2O_2 decomposition (Cohen & Hochstein, 1963), which must be rapidly removed due to its toxicity (Bai, Rodriguez, Melendez, & Cederbaum, 1999; Chance, Sies, & Boveris, 1979; Halliwell & Gutteridge, 1999; Matsumoto et al., 1999; Takagi et al., 1999).

Figs. 5 and 6 show the activities of ROS-scavenging enzymes in all the rats, before and after treatments. Results show that catalase activity of all the rats exhibited significant increase in all the rats, except for those fed the normal diet. It is interesting to note that catalase activity of rats fed 0.3% *C. asiatica* extract showed highest activity, although they were not significantly different from the rats fed either tocopherol or *C. asiatica* powder. The increase in catalase activity is crucial in counteracting the accumulation of H_2O_2 and thus suppressing the formation of the very potent and reactive $^{\cdot}OH$ radical.

In this study, significant ($p < 0.05$) decreases in superoxide dismutase activity were observed in both *C. asiatica*-fed and α-tocopherol-supplemented groups compared to either the rats fed normal diet or H_2O_2-treated rats at week 25 (end of treatments, Fig. 6). The significant decrease in

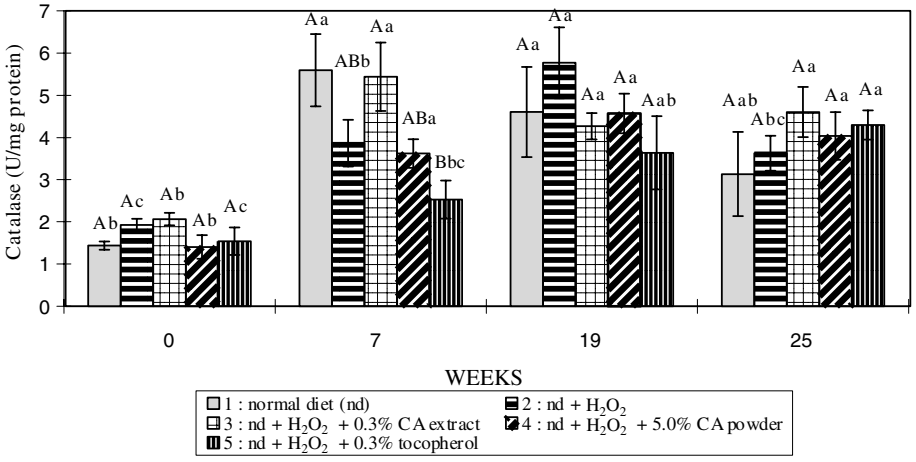

Fig. 5. Activity of catalase before (week 0) and after treatments. (A–B): Values with different letters are significantly different ($p < 0.05$) among different group treatments within a week. (a–d): Values with different letters are significantly different ($p < 0.05$) within a group at different weeks of treatments.

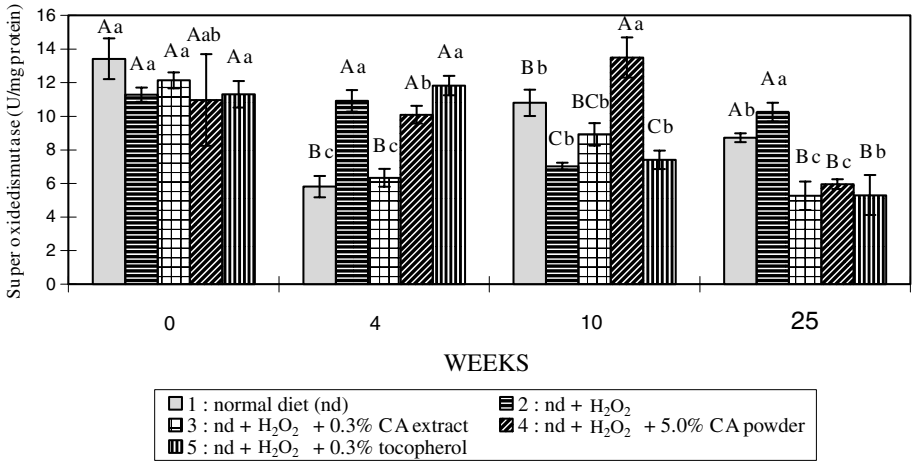

Fig. 6. Activity of superoxide dismutase before and after treatments. (A–C): Values with different letters are significantly different ($p < 0.05$) among different group treatments within a week. (a–c): Values with different letters are significantly different ($p < 0.05$) within a group at different weeks of treatments.

superoxide dismutase activity of these rats was intriguing and is probably due to the ability of antioxidant compounds in *C. asiatica* extract and powder, as well as vitamin E in the α-tocopherol supplemented group, to scavenge free radical and therefore decrease generation of ROS and lower oxidative stress. Termination of propagation of the radical by chain-breaking antioxidants (CBA) has been widely reported and is mostly considered as the secondary defence in inactivation of the propagating radical of the chain (Esterbauer, Schaur, & Zollner, 1991).

4. Conclusions

Results from this study showed that level of MDA and thus lipid peroxidation was significantly increased in normal and H_2O_2-treated rats, but this result was not shared by rats fed either *C. asiatica* or α-tocopherol. Rats fed both *C. asiatica* extract and powder had significantly lower MDA levels than had normal rats. In addition, catalase activity of rats receiving both *C. asiatica* extract and powder was found to be significantly higher than that of normal and H_2O_2-fed rats. The increase in catalase activity is crucial in counteracting the accumulation of H_2O_2 experienced by all the rats. On the other hand, it was noted that activity of superoxide dismutase was significantly lower in rats receiving both *C. asiatica* and α-tocopherol. This is intriguing and is probably indicative of the antioxidative activity of both *C. asiatica* and α-tocopherol in scavenging free radicals and thus decreasing the generation of ROS and oxidative stress of the rats concerned. These effects may be attributed to the antioxidant compounds present in *C. asiatica*, and in particular the flavonoids, quercetin, catechin and rutin, that are known to be potent antioxidants.

Acknowledgments

The authors are grateful for the research grant provided for by Government of Malaysia and for the fine facilities of the Department of Food Science, University Putra Malaysia.

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