ELSEVIER

Food Chemistry 83 (2003) 569–574

Food Chemistry

www.elsevier.com/locate/foodchem

Antioxidant action of flavonoids from *Mangifera indica* and *Emblica officinalis* in hypercholesterolemic rats

L. Anila, N.R. Vijayalakshmi*

Department of Biochemistry, University of Kerala, Kariavattom, Thiruvananthapuram- 695 581, India

Received 5 September 2002; received in revised form 18 March 2003; accepted 18 March 2003

Abstract

The oral administration of flavonoids from *Mangifera indica* and *Emblica officinalis* at a dose of 10 mg kg^{-1} body weight day^{-1} showed significant antioxidant action in cholesterol-fed experimental rats. The antioxidant activity of flavonoids was investigated on the basis of their effects on the activities of oxidative free radical scavenging enzymes, superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase, and reduced glutathione and lipid peroxide contents. The activities of free radical-scavenging enzymes were significantly elevated and lipid peroxide content was significantly decreased in flavonoid-treated hypercholesterolemic rats.

Keywords: Catalase; *Emblica officinalis*; Glutathione system; Lipid peroxides; *Mangifera indica*; Superoxide dismutase

1. Introduction

Flavonoids are phenolic compounds widely distributed in plants, with over 4000 individual substances known and the numbers constantly increasing. Plants can use them to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species caused by sunlight (Larson, 1988). Polyphenolic compounds in the diet enhance the stability of low density lipoprotein (LDL) to oxidation, and evidence exists that LDL oxidation plays a significant role in atherosclerosis and coronary heart disease (Steinberg, Parthasarathy, Carew, Khoo, & Witztum, 1989). Epidemiological studies have indicated an association between increased consumption of antioxidant-rich vegetables and fruits and a reduced risk of cardiovascular disease (Ness & Powles, 1997; Verlangieri, Kapeghian, el- Dean, & Bush, 1985). Due to the presence of the conjugated ring structures and hydroxyl groups, most phenolic compounds have the potential to function as antioxidants by scavenging the superoxide anion (Robak & Dryglewski, 1988), singlet oxygen (Husain, Cillard, & Cillard, 1987), and lipid peroxy radicals (Torel, Cillard, & Cillard, 1986) and stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species (Lewis, 1993; Shahidi & Wanasusdara, 1992). The role of antioxidants in preventing oxygen radical- and hydrogen peroxide-induced cytotoxicity and tissue damage in various human diseases is becoming increasingly recognized. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection against heart diseases and cancer is also raising interest among scientists, food manufacturers and consumers as the trend for the future is toward functional food with specific health effects (Loliger, 1991). Here we have made an attempt to elucidate the mechanism of action of the antioxidant activity of flavonoid-rich fractions from two different sources, namely *Emblica officinalis* (gooseberry) and *Mangifera indica* (mango kernel). *Emblica officinalis* is reported to have antioxidant (Bhattacharya, Chatterjee, Ghoshal, & Bhattacharya, 1999; Jose & Kuttan, 1995), hypolipidemic (Jacob, Pandey, Kapoor, & Saroja, 1988; Mathur, Sharma, Dixit, & Varma, 1996) and hypoglycemic (Anila & Vijayalakshmi, 2000) activities. In traditional medicine *Emblica officinalis* is used for various conditions, such as glucose intolerance, cerebral insufficiency and mental

* Corresponding author. Fax: +91-471-303565.
E-mail addresses: anilaleelamma@rediffmail.com (L. Anila), vijayanrlakshmi@yahoo.com (N.R. Vijayalakshmi).

0308-8146/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0308-8146(03)00155-9

disorders. There are several reports available about the traditional uses of mango kernel in various parts of the world. In Fiji, fresh mango kernel is eaten for dysentery and asthma; juice is used as a nose drop for sinus trouble (Singh, 1986). In India, dried seed powder is applied to the head to remove dandruff. The kernel starch is eaten as a famine food (John, 1984). Hot water extract of kernel is taken as an anthelmintic, aphrodisiac, laxative and tonic (Sharma, Bahga, & Srivastava, 1971). Raw mango kernels were found to contain tannins at concentrations of 56.5 g kg on a wet weight basis (Sivakanesan & Ravindran, 1996).

2. Materials and methods

2.1. Plant material and preparation of the extract

Ground-dried materials (*Emblica officinalis* and *Mangifera indica*) were extracted with hot 80% methanol (1:1, w/v) thrice (Petra, Britta, Macki, & Eckart, 1999). The combined extract was evaporated to dryness and the residue was dissolved in water and extracted successively with hexane, benzene, ethyl acetate and butanol. On estimating the levels of flavonoids in each fraction, (Eskin, Hoehn, & Frenkel, 1978), the ethyl acetate fraction was found to contain most of the flavonoids and this extract was evaporated in a vacuum. The dark brown residues (15 g each in the case of *Emblica officinalis* and *Mangifera indica*) were fractionated on a silica gel column (60 cm×40 mm), using 150 g silica gel (Sisco Research Laboratories, Bombay). The elution was performed with solvents of increasing polarity. Maximum yield was obtained in the ethyl acetate: methanol (50:50, v/v) fraction (789.4 mg) in the case of *Emblica officinalis* and the ethyl acetate (100%) fraction (954.6 mg) in the case of *Mangifera indica*. The flavonoid content was determined by the $TiCl_4$ method (Eskin et al., 1978) using quercetin as standard. These fractions were concentrated in a vacuum, reconstituted in water and used for the experiment.

2.2. Diet

As outlined in Table 1, the diet contained M/s Hindustan lever rodent chow, coconut oil (15%) and cholesterol (2%).

2.3. Animals

Male albino rats were divided into four groups of six rats each. Group I was treated as normal control, which was fed with normal laboratory diet. Group II was fed with a high fat diet and treated as high fat control, group III was fed with high fat diet + flavonoids from *Emblica officinalis* and group IV was fed with high fat diet + flavonoids from *Mangifera indica*. (The composition of the diet is given in Table 1.) Flavonoids were administered orally by gastric intubation at a dose of 10 mg kg^{-1}BW day^{-1}. Animals were kept in polypropylene cages in a room that was maintained at 28 °C and water was given ad-libitum. The duration of the experiment was 3 months. Food intake and body weight records were kept weekly. At the end of the experimental period, the rats (six in each group) were deprived of food overnight and sacrificed by euthanasia. Animals were handled in accordance with the laboratory animal welfare guidelines (Hume, 1972). Blood and tissues were collected in ice cold containers for various biochemical estimations.

Table 1
Composition of the diet

Composition of high fat diet		Composition of the rodent chow (Normal laboratory diet) (%)	
Rodent chow	83	Crude protein	21
Coconut oil	15	Ether extract (fat)	5
Cholesterol	2	Crude fibre	4
		Ash	8
		Calcium	1
		Phosphorus	0.6
		Nitrogen-free extract	53

2.4. Biochemical assays

The biochemical assays include the determination of activities of enzymes involved in the antioxidant mechanism. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assessed on the basis of colour intensity of the chromogen produced by the reduction of nitro blue tetrazolium on addition of NADH, measured at 560 nm (Kakkar, Das, & Viswanathan, 1984). Catalase (EC 1.11.1.6) was assayed by noting the decrease in extinction at 240 nm, followed by the decomposition of H_2O_2 (Maechlay & Chance, 1954). Activities of glutathione peroxidase (Gpx, EC 1.11.1.9) (Paglia & Valentine, 1967) and glutathione reductase (GR, EC 1.6.4.2) (David & Richard, 1983) were measured by following the decrease in the absorbance due to oxidation of NADPH. Glutathione-S-transferase (GST, EC 2.5.1.18) activity was assayed by measuring the increment of absorbance at 340 nm due to the formation of 2, 4-dinitrophenyl-S-glutathione from 1-chloro 2, 4-dinitrobenzene (CDNB) and GSH (Habig, Pabst & Jakoby, 1974). In addition concentrations of thiobarbituric acid reacting substances (TBARS), hydroperoxides (HP), conjugated dienes (CD) and reduced glutathione were estimated in various tissues. Malondialdehyde was identified as the product of lipid peroxidation that reacts with thiobarbituric acid forming a red coloured compound absorbing at 535 nm. Hydroperoxides were estimated by a procedure which is based on the ability

of iodide to reduce hydroperoxide and the colour intensity can be measured at 353 nm. Conjugated dienes are absorbed at 233 nm. Therefore, lipid peroxidation can be assayed by recording the increase in absorbance of extracted membrane lipids at 233 nm (John & Steven, 1978). Glutathione reacts with an excess of alloxan to produce a substance which has an absorption spectrum maximum at 305 nm (Patterson & Lazarow, 1955) in tissues and blood. Protein content was estimated in enzyme homogenate after TCA precipitation. Protein reacts with Folin-Ciocalteau reagent to give a coloured complex which can be measured at 660 nm (Lowry, Rosebrough, Farr, & Randall, 1951).

2.5. Statistical analysis

The statistical analyses were performed with the statistical software SPSS/Windows (SPSS 10.0. LNK). The results were expressed as the mean ± SEM to show variations in a group. Differences are considered significant at a P level <0.05.

3. Results

Over a period of 3 months a significantly greater weight gain was obtained for rats fed a cholesterol-containing diet compared than for normal laboratory diet-fed control rats (Table 2). The levels of malondialdehyde equivalents, hydroperoxides and conjugated dienes were significantly increased in liver, heart and kidneys of the animals in the high fat control group (Table 3). But this increase was significantly reduced to the normal control level in rats administered flavonoids from *Mangifera indica* and *Emblica officinalis*. Activities of two scavenging enzymes, SOD and catalase were significantly decreased in liver, heart and kidneys of rats fed the high fat diet (Table 4). The decrease was significantly restored to normal levels in flavonoid-treated groups. The high fat-fed rats treated with flavonoids from *Mangifera indica* showed significant elevation in the activity of SOD when compared with normal control

Table 2
Effect of flavonoids on diet intake and body weight gain of animals fed cholesterol-containing diet

Groups	Average weight per group		Weight gain
	Initial (g)	Final (g)	
I (Control)	60.0±0.95	146.0±1.24	86.0±1.58
II (High fat control)	62.0±0.88	183.0±1.83	121.0±1.60[a]
III (High fat + Emblica flavonoid)	66.0±0.76	184.0±1.89	118.0±1.54[a]
IV (High fat + Mangifera flavonoid)	65.0±1.33	185.0±1.46	120.0±1.45[a]

Average of the values from six rats in each group±SEM.
[a] $P<0.05$: Groups II, III and IV are compared with group I.

Table 3
Effect of flavonoids (10 mg kg^{-1} body weight day^{-1}) on TBARS, hydroperoxides and conjugated dienes in tissues (values expressed as nmol mg^{-1} protein) of rats fed cholesterol-containing diet

Groups	TBARS			Hydroperoxides			Conjugated dienes		
	Liver	Heart	Kidney	Liver	Heart	Kidney	Liver	Heart	Kidney
Normal control	1.25±0.022	3.03±0.045	1.54±0.030	1.21±0.060	5.34±0.082	2.32±0.045	6.75±0.149	1.79±0.080	1.65±0.110
2% Cholesterol diet	1.89±0.034[a]	4.34±0.107[a]	2.43±0.111[a]	2.21±0.134[a]	8.15±0.109[a]	3.58±0.056[a]	9.23±0.194[a]	2.70±0.083[a]	2.52±0.134[a]
2% Cholesterol + Flavonoids from *Emblica officinalis*	1.50±0.087*[a,b]	3.19±0.602*[b]	1.66±0.083*[b]	1.36±0.059*[b]	5.54±0.066*[b]	2.52±0.088*[b]	7.22±0.140[a]*[b]	1.88±0.124*[b]	1.78±0.185*[b]
2% Cholesterol + Flavonoids from *Mangifera indica*	1.40±0.092*[b]	3.20±0.033*[b]	1.77±0.106*[b]	1.48±0.095*[b]	5.60±0.073*[a,b]	2.51±0.073*[b]	7.31±0.133[a]*[b]	2.06±0.117*[b]	1.85±0.144*[b]

Average of the values from six rats in each group ±SEM.
[a] $P<0.05$: Group II, group III and group IV are compared with group I.
[b] Shows significantly similar when group III is compared with group IV.
* $P<0.05$: Group III and group IV are compared with group II.

Table 4
Effect of flavonoids (10 mg kg^{-1} body weight day^{-1}) on superoxide dismutase, catalase and glutathione content in tissues of rats fed cholesterol-containing diet

Groups	Superoxide dismutase, units[a] mg^{-1} protein			Catalase, $10^{-3}\times$units[b] mg^{-1} protein			Glutathione content, m mol 100g^{-1} wet tissue; m mol 100 ml^{-1} blood			
	Liver	Heart	Kidney	Liver	Heart	Kidney	Liver	Heart	Kidney	Blood
Normal control	8.37±0.118	14.8±0.201	15.2±0.231	69.5±0.995	12.7±0.186	29.8±0.435	487±7.1	412±6.13	230±3.52	25.8±0.405
2% Cholesterol diet	5.10±0.070[c]	10.3±0.153[c]	11.2±0.179[c]	40.3±0.521[c]	8.80±0.133[c]	21.3±0.315[c]	418±6.28[a]	357±4.45[a]	205±2.92[c]	20.4±0.307[c]
2% Cholesterol + Flavonoids from *Emblica officinalis*	8.70±0.133*	15.5±0.234*[b]	16.14±0.142*[c,d]	70.4±1.18*	14.3±0.215*[c,d]	30.4±0.486*	502±7.27*	425±5.8*	240±3.48*	26.3±0.370*
2% Cholesterol + Flavonoids from *Mangifera indica*	9.21±0.133*[b]	16.6±0.264*[b]	16.0±0.254*[c,d]	72.4±1.16*	13.8±0.214*[c,d]	32.6±0.594*[c]	495±7.15*	419±5.82*	238±3.5*	25.2±0.561*

Average of the values from 6 rats in each group ±SEM.
[a] Unit = enzyme concentration required to inhibit OD at 560 nm of chromogen produced by 50% in 1 min.
[b] Unit = velocity constant s^{-1}
[c] $P<0.05$: Group II, group III and group IV are compared with group I.
[d] Shows significantly similar when group III is compared with group IV.
* $P<0.05$: Group III and group IV are compared with group II.

rats. In the case of reduced glutathione, a significant decrease was observed in liver, heart, kidney and blood of rats fed the high fat diet (Table 4). But in high fat diet-fed rats, treated with flavonoids, the reduced glutathione (GSH) level was restored to the normal control level. Activities of GR, GPx and GST were significantly reduced in liver, heart and kidney of rats fed the high fat diet (Table 5). In the case of high fat diet-fed rats treated with flavonoids from *Emblica officinalis* and *Mangifera indica*, there was a pronounced increase of activities of these enzymes.

4. Discussion

A high fat diet brings about remarkable modifications in the antioxidant defence mechanisms of rat tissues against the process of lipid peroxidation. Several reports have shown that hyperlipidemia diminishes the antioxidant defence systems (Daniel, Biju, Devi, & Augusti, 1998), decreasing the activities of catalase and SOD and thereby elevating the lipid peroxide contents, resulting in the production of toxic intermediates. High fat induces decreases in normal activities of glutathione peroxidase and glutathione reductase enzymes and the glutathione contents in the tissues. The decreased activity of GR should normally result in a decreased concentration of reduced glutathione. The treatment with flavonoids from *Emblica officinalis* and *Mangifera indica* has elevated the levels of these parameters in tissues of experimental rats fed the high fat diet. La Casa et al. (2000) reported that rutin, a natural flavone, induced (at all tested doses) a significant increase in glutathione peroxidase activity and exerted a protective and antioxidant effect against ethanol-induced gastric lesions. Flavonoids can reduce macrophage oxidative stress by inhibition of cellular oxygenases (such as nicotinamide adenine dinucleotide phosphate), reduced form (NADPH) oxidase or by activating cellular antioxidants (such as the glutathione system) (Fuhrman & Aviram, 2001). Pignol et al. (1988) reported antilipoperoxidative properties of flavonoids purified from *Ginko biloba* extract. Antioxidant activities of flavonoids from *Solanum melongen*a and *Solenostemon rotendifolius* have been reported from this laboratory (Sandhya & Vijayalakshmi, 2001; Sudheesh, Sandya, Koshy, & Vijayalakshmi, 1999). Recent studies on the antioxidant properties of flavonoids reveal their stimulatory action on antioxidative enzymes (Miyake, Yamamoto, Tsujihara, & Osawa, 1998; Nagata, Takekoshi, Takagi, Honma, & Watanabe, 1999).

In the present study, the activities of SOD and catalase in tissues of rats fed the cholesterol-containing diet were significantly decreased when compared to the normal laboratory diet-fed control rats. The administration of flavonoids from *Emblica officinalis* and *Mangifera*

Table 5
Effect of flavonoids (10 mg kg^{-1} body weight day^{-1}) on the activities of glutathione peroxidase, glutathione reductase and glutathione-S-transferase in tissues of rats fed cholesterol-containing diet

Groups	Glutathione reductase, $10^{-2}\times$units[a] mg^{-1} protein			Glutathione peroxidase, units[a] mg^{-1} protein			Glutathione-S-transferase (μmol of thioester formed min^{-1} mg^{-1} protein)		
	Liver	Heart	Kidney	Liver	Heart	Kidney	Liver	Heart	Kidney
Normal control	5.46±0.130	4.55±0.090	7.35±0.107	0.185±0.004	0.684±0.010	0.282±0.007	7.88±0.190	7.8±0.109	6.89±0.091
2% Cholesterol diet	3.26±0.049[b]	3.42±0.055[b]	5.52±0.083[b]	0.129±0.008[b]	0.526±0.022[b]	0.182±0. 005[b]	4.30±0.124[a]	4.87±0.071[b]	1.84±0.026[b]
2% Cholesterol + Flavonoids from *Emblica officinalis*	5.58±0.128*	4.81±0.088*	7.23±0.104*[c]	0.185±0.006*	0.591±0.008*[c]	0.234±0.018*[b,c]	7.21±0.250*[b,c]	6.93±0.101*[b,c]	6.24±0.087*
2% Cholesterol + Flavonoids from *Mangifera indica*	5.30±0.157*	4.85±0.178*	6.88±0.176*[b,c]	0.188±0.006*	0.600±0.018*[c]	0.246±0.015*[c]	6.91±0.300*[b,c]	7.42±0.112*[c]	5.64±0.082*

Average of the values from 6 rats in each group ±SEM.
[a] Unit = μmol NADPH oxidized min^{-1}.
[b] $P<0.05$:Group II, group III and group IV are compared with group I.
[c] Shows significantly similar when group III is compared with group IV.
* $P<0.05$: Group III and group IV are compared with group II.

indica to the rats fed the cholesterol-containing diet showed significant elevation in the activities of these two enzymes. Recent reports show that TBARS in the gastric mucosa, an index of lipid peroxidation, were increased by ethanol injury, but the increase was inhibited by the administration of 200 mg/kg quercetin, a natural flavone, through decrease of reactive oxygen metabolites (Martin et al., 1998). Administration of flavonoids from *Emblica officinalis* and *Mangifera indica* at a dose of 10 mg kg^{-1}BW day^{-1} could effectively lower the levels of lipid peroxides and MDA equivalents in high fat-fed rats. Further research is in progress, aimed at characterizing the flavonoid fraction and isolation of the active flavonoid compounds.

Acknowledgements

Financial assistance received from the Indian Council of Medical Research, New Delhi in the form of SRF is acknowledged with thanks.

References

Anila, L., & Vijayalakshmi, N. R. (2000). Beneficial effects of flavonoids from Sesamum indicum, *Emblica officnalis* and *Momordica charantia*. *Phytotherapy Research*, *14*, 1–4.

Bhattacharya, A., Chatterjee, A., Ghoshal, S., & Bhattacharya, S. K. (1999). Antioxidant activity of tannoid principles of *Emblica officinalis* (amla). *Indian Journal of Experimental Biology*, *37*(7), 676–680.

David, M., & Richard, J. S. (1983). Glutathione reductase. In H. U. Bergmeyer (Ed.), *Methods of enzymatic analysis* (pp. 258–265). Weinheim, Florida: Verlag Chemie.

Daniel, R. S., Biju, C. M., Devi, K. S., & Augusti, K. T. (1998). Antioxidant effect of two flavonoids from the bark of *Ficus bengalensis* Linn in hyperlipidemic rats. *Indian Journal of Experimental Biology*, *36*, 902–906.

Eskin, N. A. M., Hoehn, E., & Frenkel, C. J. (1978). A simple and rapid quantitative method for total phenols. *Journal of Agricultural & Food Chemistry*, *26*, 973–974.

Fuhrman, B., & Aviram, M. (2001). Flavonoids protect LDL from oxidation and attenuate atherosclerosis. *Current Opinion in Lipidology*, *12*(1), 41–48.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione- S-Transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, *249*, 7130–7139.

Hume, C. W. (1972). *The UFAW handbook on the care and management of laboratory animals*. Edinburgh, London: Churchill Livingstone.

Husain, S. R., Cillard, J., & Cillard, P. (1987). Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry*, *26*, 2489–2491.

Jacob, A., Pandey, M., Kapoor, S., & Saroja, R. (1988). Effect of the Indian gooseberry (amla) on serum cholesterol levels in men aged 35–55 years. *European Journal of Clinical Nutrition*, *42*(11), 939–944.

John, A. B., & Steven, A. D. (1978). Microsomal lipid peroxidation. In S. Fleischer, & L. Packer (Eds.), *Methods in enzymology* (pp. 302–310). New York: Academic Press.

John, D. (1984). One hundred useful raw drugs of the Kani Tribes of Trivandrum Forest Division, Kerala, India. *International Journal of Crude Drug Research*, *22*(1), 17–39.

Jose, J. K., & Kuttan, R. (1995). Antioxidant activity of *Emblica officinalis*. *Journal of Clinical Biochemistry & Nutrition*, *19*(2), 63–70.

Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry & Biophysics*, *21*, 130–132.

La Casa, C., Villegas, I., Alarcon-de-la-Lastra, C., Motilva, V., & Martin Calero, M. J. (2000). Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. *Journal of Ethnopharmacology*, *71*(1-2), 45–53.

Larson, R. A. (1988). The antioxidants of higher plants. *Phytochemistry*, *27*, 969–972.

Lewis, N. G. (1993). Antioxidants in higher plants. In R. G. Alscher, & J. L. Hess (Eds.), *Plant phenolics* (pp. 135–169). Boca Raton: CRC Press.

Loliger, J. (1991). The use of antioxidants in food. In O. I. Aruoma, & B. Halliwell (Eds.), *Free radicals and food additives* (pp. 129–150). London: Taylor and Francis.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Maechlay, A. C., & Chance, B. (1954). The assay of catalase and peroxidase. In D. Glick (Ed.), *Methods of biochemical analysis* (pp. 357–424). New York: Interscience Inc.

Martin, M. J., La Casa, C., Alarcone, de.-la., Lastra, C., Cabeza, J., Villegas, I., & Motilva, V. (1998). Antioxidant mechanisms involved in gastroprotective effects of quercetin. *Z. Naturforsch.*, *53*(1-2), 82–88.

Mathur, R., Sharm, A., Dixit, V. P., & Varma, M. (1996). Hypolipidemic effect of fruit juice of *Emblica officinalis* in cholesterol fed rabbits. *Journal of Ethnopharmacology*, *50*(2), 61–68.

Miyake, Y., Yamamoto, K., Tsujihara, N., & Osawa, T. (1998). Protective effects of lemon flavonoids on oxidative stress in diabetic rats. *Lipids*, *33*(7), 689–695.

Nagata, H., Takekoshi, S., Takagi, T., Honma, T., & Watanabe, K. (1999). Antioxidative action of flavonoids, quercetin and catechin, mediated by the activation of glutathione peroxidase. *Tokai J. Exp. Clin. Med.*, *24*(1), 1–11.

Ness, A. R., & Powles, J. W. (1997). Fruit and vegetables, and cardiovascular disease: a review. *International Journal of Epidemiology*, *26*, 1–13.

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, *70*, 158–161.

Patterson, J. E., & Lazarow, A. (1955). Determination of Glutathione. In D. Glick (Ed.), *Methods of biochemical analysis* (pp. 259–278). New York: Interscience Inc.

Petra, M., Britta, T., Macki, K., & Eckart, E. (1999). Flavonoid sulfates from the Convolvulaceae. *Phytochemistry*, *50*, 267–271.

Pignol, B., Etienne, A., Crastesde-Panlet, C., Deby, J. M., Mencia, H., & Braquet, P. In V. Cody, E. Middleton, & J. B. Harbone (Eds.), *Plant flavonoids in biology and medicine* (pp. 183–186). New York: Alan R Liss Inc.

Robak, J., & Dryglewski, R. J. (1988). Flavonoids are scavengers of superoxide anion. *Biochemical Pharmacology*, *37*, 83–88.

Sandhya, C., & Vijayalakshmi, N. R. (2001). Antioxidant activity of flavonoids from *Solenostemon rotendifolius*. *Journal of Nutraceuticals, Functional & Medicinal Foods*, *3*(2), 55–66.

Shahidi, F., & Wanasusdara, J. P. K. P. D. (1992). Phenolic antioxidants. *Critical Reviews of Food Science & Nutrition*, *32*, 67–103.

Sharma, L. D., Bahga, H. S., & Srivastava, P. S. (1971). In vitro anthelmintic screening if indigenous medicinal plants against *Haemonchus contortus* (Rudolphi, 1803) cobbold, 1898 of sheep and goats. *Indian Journal of Animal Research*, *5*(1), 33–38.

Singh, Y. N. (1986). Traditional medicine in Fiji. Some herbal folk cures used by Fiji Indians. *Journal of Ethnopharmacology*, *15*(1), 57–88.

Sivakanesan, R., & Ravindran, V. (1996). The nutritive value of Mango seed kernels for starting chicks. *Journal of Science of Food & Agriculture*, *71*(2), 245–250.

Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., & Witztum, J. L. (1989). Beyond cholesterol: Modification of low density lipoproteins that increase its atherogenecity. *New England Journal of Medicine*, *320*, 915–924.

Sudheesh, S., Sandhya, C., Koshy, A. S., & Vijayalakshmi, N. R. (1999). Antioxidant activity of flavonoids from *Solanum melongena*. *Phytotherapy Research*, *13*, 393–396.

Torel, J., Cillard, J., & Cillard, P. (1986). Antioxidant activity of flavonoids and reactivity with peroxy radical. *Phytochemistry*, *25*, 383–385.

Verlanier, A. J., Kapeghian, J. C., el- Dean, S., & Bush, M. (1985). Fruit and vegetable consumption and cardiovascular disease mortality. *Medical Hypotheses*, *16*, 7–15.