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RESEARCH ARTICLE

Protective effect of medicinal plant extracts on biomarkers of oxidative stress in erythrocytes

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Abstract

Plants are universally recognized as a vital part of the world's natural heritage and up to 80% of the population rely on plants for their primary healthcare. Varieties of medicinal plants are recognized as a source of natural antioxidants that can protect from oxidative stress, thus playing an important role in chemoprevention of diseases. In the present investigation, 22 extracts from different parts of eight medicinal plants (*Punica granatum* Linn. (Punicaceae), *Caesalpinia bonducella* Flem. (Fabaceae), *Hibiscus subdariffa* Linn. [(Malvaceae), *Moringa oleifera* Lam. (Moringaceae), *Garcinia indica* Linn. (Clusiaceae), *Emblica officinalis* Gaertn. (Euphorbiaceae), *Momordica charantia* Linn. (Cucurbitaceae), and *Matricaria chamomilla* Linn. (Asteraceae)] were screened for their protection against oxidative stress in erythrocytes induced by hydrogen peroxide (2 mM) and *tert*-butyl hydroperoxide (0.01 mM). The effect was also compared with known antioxidants and flavonoids. Subjecting erythrocytes to oxidative stress by incubation with hydrogen peroxide and *tert*-butyl hydroperoxide caused a significant alteration in reduced glutathione (GSH) and malondialdehyde (MDA) concentration compared to the control. Our results show that medicinal plant extracts protect erythrocytes from hydrogen peroxide and *tert*-butyl hydroperoxide induced oxidative stress; known antioxidant (vitamin C, E, and β-carotene) and flavonoid (quercetin) also showed a similar protective effect. Our observations may, in part, suggest the use of the spent/waste parts of medicinal plants. This could be an economically viable source of natural and potent antioxidants effective against complications arising from oxidative stress. The results may also improve the ethanopharmacological knowledge of medicinal plants.

Keywords: *Medicinal plants; glutathione (GSH); malondialdehyde (MDA); oxidative stress; antioxidant; hydrogen peroxide; tert-butyl hydroperoxide*

Introduction

Plants, universally recognized as a vital part of world's natural heritage, supply our basic needs such as food, fibers, fuel, and many important medicines. The traditional systems of medicine such as Ayurveda, Siddha, Unani, homeopathy, and folklore of various countries, depend on plants for their medicinal preparation. The medicinal properties of plants are mainly attributed to the presence of flavonoids, coumarins, phenolic acids, and antioxidant micronutrients such as Cu, Zn and Mn (Repetto & Llesuy, 2002; Seth & Sharma, 2004). Varieties of medicinal plants are recognized as a source of natural antioxidants that can protect from oxidative stress and thus play important role in chemoprevention of diseases (Lee et al., 2003; Katalinic et al., 2006).

Oxidative stress arising from free radicals like reactive oxygen species (ROS) now appears to be a fundamental mechanism underlying degenerative diseases such as diabetes, viral infection, autoimmune pathologies and probably aging. Evidence suggests that ROS can be scavenged through chemoprevention utilizing antioxidant compounds present in foods and medicinal plants (Surh, 1999; Finkel & Holbrook, 2000). Antioxidant principles

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from herbal resources are multifaceted in their effects and their proper intake helps us to fight against various metabolic imbalances. It has been reported that nutritional intervention to increase intake of phyto-antioxidants may reduce the threat of free radicals (Ng et al., 2000; Arora et al., 2003). Plants play a significant role in maintaining human health and improving the quality of human life. They serve humans as valuable components of food, cosmetics, dyes, and medicines. The World Health Organization estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary healthcare needs, and most of this therapy involves the use of plant extracts and their active components (Winston, 1999; Tripathi et al., 2007).

Erythrocytes are well equipped to degrade reactive oxygen species via the actions of superoxide dismutase that converts O_2 into H_2O_2, which is further catabolized by catalase and glutathione peroxidase. Mammalian cells contain 1-10 mM cytosolic glutathione (GSH) under normal physiological conditions, depending on the cell type and metabolic factors. GSH represents approximately 95% of total non-protein thiols and is the main modulator of the cellular redox environment. The cytoplasmic ratio of reduced to oxidized glutathione (GSH/GSSG) of approximately 100/1, maintains the cysteine residues of intracellular proteins in the reduced form. GSSG generation from GSH can be favored during mild oxidative stress conditions. The oxidation of GSH to GSSG can dramatically change the GSH/GSSG ratio and consequently the redox status within the cells. As a result, the thiol group of cytosolic proteins is modified by the reversible formation of protein GSH mixed disulfides, a process known as *S*-glutathionylation (Davies & Goldberg, 1987; Rohn et al., 1998; Di Simplicio et al., 1998; Telci et al., 2000). Due to oxidative stress, polyunsaturated fatty acid of erythrocyte membrane is damaged resulting in a steep increase in malondialdehyde (MDA) concentration, a biomarker currently used for studying oxidation of lipids under different conditions (Pradhan et al., 1990; Konukoglu et al., 1998; Lopez-Revuelta et al., 2005).

In the present study, erythrocytes are used as a model system for studying oxidative damage and its pathophysiology. Twenty-two extracts prepared from different parts of eight medicinal plants - *Punica granatum* Linn. (Punicaceae), *Caesalpinia bonducella* Flem. (Fabaceae), *Hibiscus subdariffa* Linn. (Malvaceae), *Moringa oleifera* Lam. (Moringaceae), *Garcinia indica* Linn. (Clusiaceae), *Emblica officinalis* Gaertn. (Euphorbiaceae), *Momordica charantia* Linn. (Cucurbitaceae) and *Matricaria chamomilla* Linn. (Asteraceae) - were screened for their protection against oxidative stress in erythrocytes induced by hydrogen peroxide and *tert*-butyl hydroperoxide and the effect was compared with standard antioxidants like vitamin E, vitamin C, β-carotene, and quercetin (a flavonoid). Evaluation of GSH and MDA concentration was done and the protective effect of antioxidants and plant extracts in erythrocytes subjected to oxidation by hydrogen peroxide/*tert*-butyl hydroperoxide were compared and recorded. The results demonstrated that some of the extracts showed significant protection against hydrogen peroxide/*tert*-butyl hydroperoxide-induced oxidative stress.

Materials and methods

Collection of plant material and extract preparation

The plant materials were collected either from the research farm of CIMAP (Central Institute of Medicinal and Aromatic Plants, Lucknow) or purchased from the local market during January to September 2006. The plant materials were authenticated by J. Singh, S.P. Jain, and S.C. Singh, and the voucher specimens were deposited in Gyan Surabhi (Table 1). Extracts were prepared as described previously with slight modification (Luqman et al., 2005). Plant samples were washed and shade-dried at 40°C. Then, dried plant samples were extracted with ethanol (99.5%, Merck Specialities, Mumbai, India) and/or distilled water. Three-step extractions were done and extracts were filtered and concentrated in a rotary evaporator (Buchi, Flavil, Switzerland) at 45°C. All the extracts were dried at 40°C in a hot air oven and stored at 4°C. Plant extract were prepared by dissolving 100mg of extracts either in dimethyl sulfoxide (DMSO 99.5%) or in methanol (99.8%; Merck Specialities, Mumbai, India). The final concentration of DMSO and/or methanol was always < 0.05% in the experimental solution.

Table 1. List of plants and plant parts.

Name of the Plants, Authority and Family	Parts Used	Type of Extracts	Voucher Specimen Number
Emblica officinalis Linn. (Punicaceae)	Fruit	Alcohol & Aqueous	9071
Momordica charantia Linn. (Cucurbitaceae)	Fruit	Alcohol & Aqueous	10065
Moringa oleifera Lam. (Moringaceae)	Leaf, Fruit (Pod)	Alcohol & Aqueous	9076
Matricaria chamomilla Linn. (Asteraceae)	Flower	Alcohol & Aqueous	9075
Hibiscus subdariffa Linn. (Malvaceae)	Sepal	Alcohol & Aqueous	12008
Caesalpinia bonducella Flem. (Fabaceae)	Seed, Seed Coat	Alcohol & Aqueous	9084
Garcinia indica Linn. (Clusiaceae)	Fruit	Alcohol & Aqueous	9085
Punica granatum Linn. (Punicaceae)	Seed, Peel	Alcohol & Aqueous	9078

Collection of blood and isolation of packed erythrocytes

Retino-orbital blood from healthy male mice (aged 120–145 days, bodyweight 35 ± 6 g, hemoglobin 14 ± 3 g/mL) was collected for experiments using heparin (10 units/mL) as the anti-coagulant. The collected blood was stored at 4°C and was used for experiments within four hours of collection (Luqman & Rizvi, 2006; Rizvi & Luqman, 2002). Blood samples were centrifuged at 4°C for 10 min at 100 g to remove plasma and buffy coat and the isolated erythrocytes were washed 3–4 times with 0.154 M NaCl, and finally packed erythrocytes were obtained. The animals were authenticated and approved by the Institutional Animal Ethical Committee (IAEC) of the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow.

Estimation of reduced glutathione concentration

The reduced glutathione concentration in erythrocytes was estimated using the standard method of Beutler et al. (1963) as reported previously (Rizvi & Luqman, 2002) with slight modification. To 100 μL of packed RBC, 900 μL of phosphate solution was added. Tubes were centrifuged at 5,000 rpm for 5 min and supernatant was discarded. To 100 μL of RBC (pellet), 100 μL of phosphate solution was added. Next, 900 μL of distilled water was added to the suspension, followed by the addition of 1.5 mL precipitation solution, and the tubes were centrifuged at 5,000 rpm for 3 min. To the 50 μL of supernatant, 200 μL phosphate solution and 25 μL of freshly prepared DTNB were added. This method is based on the ability of the sulfhydryl group to reduce 5, 5´-dithiobis 2-nitro benzoic acid (DTNB) and form a yellow-colored anionic product whose absorbance is measured at 412 nm. The concentration of GSH is expressed as μmol/mL of packed erythrocytes and was determined from a standard plot.

Estimation of malondialdehyde concentration

Erythrocyte malondialdehyde formed during lipid peroxidation was measured according to the method of Esterbauer and Cheeseman (1990) as described earlier (Luqman & Rizvi, 2006). Packed erythrocytes (200 μL) were suspended in 3 mL of PBS-glucose solution (pH 7.4). To 1 mL of the suspension, 1 mL of 10% TCA was added. Centrifugation was done for 5 min at 5,000 rpm. To 1 mL of supernatant, 1 mL of 0.67% TBA in 0.05 M NaOH was added. Tubes were kept in a boiling water bath for 20 min at a temperature greater than 90°C and then cooled. Absorbance was measured at 532 nm (OD_1) and 600 nm (OD_2) against a blank. The net optical density (OD) was calculated after subtracting absorbance at OD_2 from that of OD_1. The concentration of MDA in erythrocytes was determined from a standard plot and expressed in terms of nmol/mL of packed erythrocytes.

In vitro experiments with plant extracts, vitamins, quercetin and induction of oxidative stress

The protective effect of plant extracts on erythrocyte GSH and MDA concentration was investigated as follows: blood samples were washed three times with 5 mM phosphate buffer saline containing glucose (pH 7.4). Erythrocytes were then suspended in 10 volumes of phosphate buffer saline. The protective effect of plant extracts (100 μg/mL), vitamins (10 μg/mL) and quercetin (10 μg/mL) were evaluated by pre-incubating erythrocytes separately for 30 min at 37°C. Oxidative stress was induced *in vitro* by incubating erythrocytes with hydrogen peroxide (2 mM)/*tert*-butyl hydroperoxide (0.01 mM) in the above experiments. The concentration of hydrogen peroxide and *tert*-butyl hydroperoxide used in the present study to induce oxidative stress was in the range of concentrations used in previously published reports (Mueller et al., 1997; Mendiratta et al., 1998; Domanski et al., 2005; Iglesias & Catala, 2005; Rizvi et al., 2005; Rizvi & Kumar, 2005).

Statistical analysis

Statistical analysis of the data obtained was performed by employing mean ± standard deviation (SD). A Student's *t*-test was used to make a statistical comparison between two-tailed paired groups. A comparison was done with the control group versus oxidized group and oxidized group versus extract treated group. The significance levels were set at $p < 0.001$, < 0.01 and/or < 0.05.

Results and discussion

Erythrocytes are unique cells well equipped to degrade reactive oxygen species through superoxide dismutase, catalase, glutathione/glutathione peroxidase, methemoglobin reductase, and membrane bound α-tocopherol (Halliwell & Gutteridge, 1986). Induction of oxidative stress following incubation with hydrogen peroxide and *tert*-butyl hydroperoxide resulted in a decrease in GSH concentration (57.8% and 49.4% respectively) in erythrocytes as compared to control (Figure 1). The ability of standard antioxidant to maintain reduced GSH levels in erythrocytes was found to be in the following order: β-carotene > quercetin > vitamin C > vitamin E (hydrogen peroxide), β-carotene > vitamin C > quercetin = vitamin E (*tert*-butyl hydroperoxide).

β-Carotene was found to be a more potent antioxidant, which is effective against oxidative stress generated

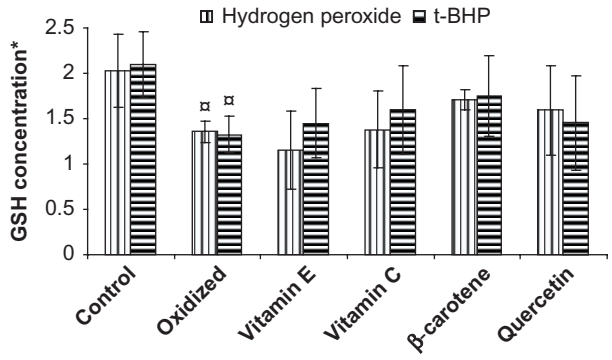

Figure 1. Protective effect of vitamins and flavonoid on erythrocyte reduced glutathione concentration stressed with hydrogen peroxide/*tert*-butyl hydroperoxide. * Concentration of GSH is expressed as μmol/ml of packed erythrocytes; Values are mean ± SD of three experiments in replicates; ¤ $p < 0.01$.

by *tert*-butyl hydroperoxide and hydrogen peroxide. Our finding corroborates with previous reports on protection of β-carotene against radiation-induced oxidative stress which is attributed to the quenching of singlet oxygen (Gerster, 1993). In addition, β-carotene has also been reported to scavenge a variety of free radical species (Krinsky & Deneke, 1982). Epidemiological studies have shown a link between dietary carotenoids and the reduced incidence of certain disease, including cancers (Manda & Bhatia, 2003). However, few reports (Omenn et al., 1996) have shown β-carotene supplementation has little or no beneficial effect. The presence of plant extracts in the incubation medium protected the erythrocytes from hydrogen peroxide and *tert*-butyl hydroperoxide-induced oxidative stress, as evidenced by maintaining GSH concentration; known antioxidants also showed a similar effect (Table 2; Figure 1). The protective effect of medicinal plant extract was found to be significant at 100 μg/mL. The alcoholic and aqueous seed extract of *Caesalpinia bonducella* showed maximum protection of GSH concentration against the stress induced by hydrogen peroxide whereas when the stress was induced by t-BHP, the aqueous extract of *Punica granatum* seed showed maximum protection. Lower concentration of medicinal plant extracts did not show significant protection of GSH concentration in erythrocytes against the stress induced by hydrogen peroxide and *tert*-butyl hydroperoxide (data not shown).

Reduced glutathione is a major intracellular non-protein sulfhydryl compound having many biological functions, including maintenance of membrane protein -SH groups in the reduced form, the oxidation of which can otherwise cause altered cellular structure and function (Reglinski et al., 1988; Di Simplicio et al., 1996). Membrane -SH group oxidative damage may be an important molecular mechanism inducing changes in the membrane microelasticity or whole cell deformability of erythrocytes under conditions of physiological and pathological oxidative stress. The importance of protein -SH groups as critical buffers to maintain the intracellular redox state has been suggested by the report of protein thiol having reactivity similar to or greater than that of GSH (Wang et al., 1999).

The erythrocyte membrane is prone to lipid peroxidation under oxidative stress that leads to the formation of MDA, a biomarker used for studying the oxidation of lipids under different conditions. Subjecting erythrocytes to oxidative stress by incubating them with hydrogen peroxide and/or *tert*-butyl hydroperoxide caused an increase in MDA (110/76%) concentration above the basal level in erythrocytes. MDA concentration did not increase in vitamin C (10 μg/mL) treated erythrocytes stressed with hydrogen peroxide. The ability of standard antioxidant to maintain MDA concentration in erythrocytes was found to be in the following order: vitamin C > quercetin > β-carotene > vitamin E. Our results suggest that cellular injury caused by hydrogen peroxide through generation of superoxide ion and OH radical can be checked by increasing vitamin C dosage and even reduced GSH level can be efficiently replenished in erythrocytes. Vitamin C reacts with the α-tocopheroxyl radical to regenerate α-tocopherol (Buettner, 1993). Further recycling of vitamin C occurs from the ascorbate free radical (one-electron oxidized form) and dehydroascorbic acid (two-electron oxidized form), respectively (May et al., 1998). Ascorbate free radical can be reduced by NADH-dependent cytochrome b5 reductases or thioredoxin reductase (May et al., 2004). Dehydroascorbic acid can be reduced directly by GSH and GSH-dependent enzymes (Washburn & Wells, 1999), or by NADPH-dependent dehydroascorbate reductase (Del Bello et al., 1994).

Quercetin (10 μg/mL) was found to be most efficient in protecting the lipid peroxidation, thereby limiting MDA formation in erythrocytes stressed with *tert*-butyl hydroperoxide. This suggests that quercetin may inhibit peroxynitrite radical formation that usually increases due its presence and even normal activity of Ca^{++}-ATPase pump can be attained. Agents like superoxide ion and hydrogen peroxide that are produced due to interaction of *tert*-butyl hydroperoxide with Hb and MetHb can be decreased in the presence of quercetin and thus cellular injury can be checked (Deliconstantinos et al., 1996). Protective effects of quercetin against hydrogen peroxide-induced DNA damage have been reported in previous studies (Musonda & Chipman, 1998; Blasiak et al., 2002; Peng & Kuo, 2003). It was also found that quercetin efficiently protects hydrogen peroxide-induced DNA damage in RLE cells (Boots et al., 2007). The plant extracts, when present in the incubation medium at a concentration of 100 μg/mL, were found to protect the erythrocytes from the damage induced by hydrogen peroxide and

Table 2. Protective effects of plant extracts on reduced glutathione and malondialdehyde concentration in erythrocytes stressed by hydrogen peroxide and *tert*-butyl hydroperoxide.

	GSH concentration[1]		MDA concentration[2]	
Plant extracts	H_2O_2	t-BHP	H_2O_2	t-BHP
Emblica officinalis (Fruit: Aqs)	0.885 ± 0.36	1.29 ± 0.75	0.057 ± 0.01	0.05 ± 0.02
Emblica officinalis (Fruit: Alc)	1.462 ± 0.40	2.07 ± 0.45	0.042 ± 0.01**	0.053 ± 0.008
Hibiscus subdariffa (Sepal: Aqs)	1.303 ± 0.55	1.98 ± 0.37***	0.058 ± 0.01	0.1395 ± 0.15
Hibiscus subdariffa (Sepal: Alc)	1.889 ± 1.3	1.905 ± 0.34	0.0309 ± 0.01	0.041 ± 0.046
Punica granatum (Peel: Aqs)	2.13 ± 0.90	1.939 ± 0.17**	0.035 ± 0.003	0.047 ± 0.01***
Punica granatum (Peel: Alc)	1.65 ± 0.54	2.209 ± 0.81	0.039 ± 0.002	0.048 ± 0.021
Punica granatum (Seed: Alc)	1.42 ± 1.01	1.956 ± 0.36	0.053 ± 0.007**	0.041 ± 0.007**
Punica granatum (Seed: Aqs)	1.15 ± 0.40	2.37 ± 0.32*	0.047 ± 0.008*	0.066 ± 0.026
Momordica charantia (Fruit: Alc)	1.83 ± 1.1	2.06 ± 0.81	0.054 ± 0.025	0.1205 ± 0.05
Momordica charantia (Fruit: Aqs)	1.19 ± 0.62	2.30 ± 0.30**	0.075 ± 0.06	0.142 ± 0.17
Matricaria chamomilla (Flower: Alc)	1.12 ± 0.38	1.78 ± 0.45	0.074 ± 0.012	0.313 ± 0.24
Matricaria chamomilla (Flower: Aqs)	1.47 ± 0.42	1.962 ± 0.18**	0.0645 ± 0.005	0.127 ± 0.07
Moringa oleifera (Pod: Alc)	1.22 ± 0.23	1.82 ± 0.51	0.05 ± 0.007***	0.091 ± 0.03
Moringa oleifera (Pod: Aqs)	1.96 ± 0.61	1.97 ± 0.2**	0.05 ± 0.013***	0.077 ± 0.01
Moringa oleifera (Leaf: Alc)	2.14 ± 0.59	1.97 ± 0.41***	0.06 ± 0.01	0.128 ± 0.09
Moringa oleifera (Leaf: Aqs)	1.94 ± 0.49	1.843 ± 0.31	0.063 ± 0.009	0.08 ± 0.02
Garcinia indica (Fruit: Alc)	1.92 ± 0.46	1.86 ± 0.43	0.057 ± 0.012	0.051 ± 0.001
Garcinia indica (Fruit: Aqs)	1.61 ± 0.37	1.94 ± 0.26***	0.093 ± 0.031	0.55 ± 0.01
Caesalpinia bonducella (Seed Coat: Alc)	1.92 ± 1.04	1.2 ± 0.85	0.037 ± 0.025*	0.54 ± 0.04
Caesalpinia bonducella (Seed Coat: Aqs)	2.61 ± 0.78**	0.522 ± 2.5	0.060 ± 0.025	0.049 ± 0.05
Caesalpinia bonducella (Seed: Aqs)	2.75 ± 0.84**	0.98 ± 0.62	0.058 ± 0.031	0.116 ± 0.03
Caesalpinia bonducella (Seed: Alc)	2.75 ± 1.34***	0.6545 ± 0.87	0.045 ± 0.028*	0.252 ± 0.21
Control (without oxidation)	2.095 ± 0.44	2.039 ± 0.014	0.04996 ± 0.005	0.0488 ± 0.015
Positive control (with oxidation)	0.915 ± 0.16*	1.0314 ± 0.67**	0.1052 ± 0.048**	0.086 ± 0.014***

Aqs:Aqueous extract(s), Alc:Alcohol extract(s).
[1]GSH concentration is expressed as µmol/ml of packed erythrocytes.
[2]Malondialdehyde concentration is expressed as nmol/ml of packed erythrocytes.
*p < 0.001, **p < 0.01, ***p < 0.05.

Table 3. Ascending order of effectiveness of medicinal plant extract providing protection to erythrocytes stressed by hydrogen peroxide and *tert*-butyl hydroperoxide.

Parameter (s)	Condition (s) of stress	Order of Protection by plant extract (s)
GSH concentration	Hydrogen peroxide	*Caesalpinia bonducella* (seed: Alc & Aqs) > *Caesalpinia bonducella* (seed coat: Aqs) > *Moringa oleifera* (leaf: Alc) > *Punica granatum* (peel: Aqs) > *Punica granatum* (seed: Alc) > *Moringa oleifera* (pod: Aqs) > *Moringa oleifera* (leaf: Aqs) > *Garcinia* (fruit: Alc) > *Caesalpinia bonducella* (seed coat: Alc) > *Hibiscus subdariffa* (sepal: Alc)
	tert-butyl hydroperoxide	*Punica granatum* (seed: Aqs) > *Punica granatum* (peel: Alc) > *Momordica charantia* (fruit: Aqs) > *Emblica officinalis* (fruit: Alc) > *Momordica charantia* (fruit: Alc) > *Hibiscus subdariffa* (sepal: Aqs) > *Moringa oleifera* (pod: Aqs) > *Moringa oleifera* (leaf: Alc) > *Garcinia* (fruit: Aqs) > *Matricaria chamomilla* (flower: Aqs)
MDA concentration	Hydrogen peroxide	*Hibiscus subdariffa* (sepal: Alc) > *Punica granatum* (peel: Aqs) > *Caesalpinia bonducella* (seed coat: Alc) > *Punica granatum* (peel:Alc) > *Emblica officinalis* (fruit: Alc) > *Caesalpinia bonducella* (seed: Alc) > *Punica granatum* (seed: Aqs) > *Moringa oleifera* (pod: Aqs) > *Moringa oleifera* (pod: Alc) > *Punica granatum* (seed: Alc)
	tert-butyl hydroperoxide	*Hibiscus subdariffa* (sepal: Alc) > *Punica granatum* (seed: Alc) > *Punica granatum* (peel: Aqs & Alc) > *Caesalpinia bonducella* (seed coat: Alc) > *Emblica officinalis* (fruit: Aqs) > *Garcinia* (fruit: Alc) > *Emblica officinalis* (fruit: Alc) > *Punica granatum* (seed: Aqs) > *Moringa oleifera* (pod: Aqs)

tert-butyl hydroperoxide (Table 2; Figure 2). The alcoholic extract of *Hibiscus subdariffa* sepal was able to protect the erythrocytes from oxidative stress induced by both hydrogen peroxide and *tert*-butyl hydroperoxide, to the maximum extent among all other plant extracts (Table 3).

Oxidation with hydrogen peroxide and *tert*-butyl hydroperoxide resulted in increased erythrocyte MDA concentrations and lower level of intracellular GSH. Alteration in the normal level of MDA and GSH concentration in stressed erythrocytes are indicators of an increased pro-oxidant/antioxidant ratio compared with normal

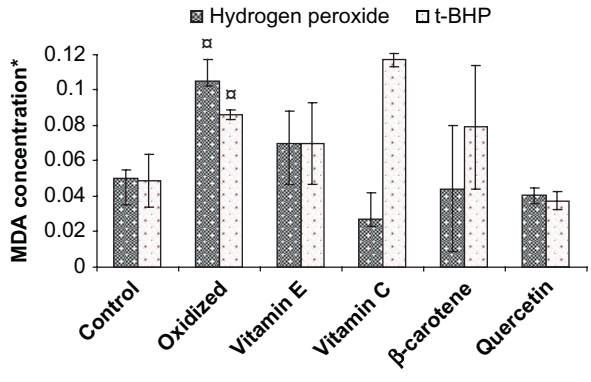

Figure 2. Protective effect of vitamins and flavonoid on erythrocyte malondialdehyde concentration stressed with hydrogen peroxide/*tert*- butylhydroperoxide. *Concentration of MDA is expressed as nmol/ml of packed erythrocytes; Values are mean ± SD of three experiments in replicates; ¤ $p < 0.01$.

erythrocytes. Our results are consistent with those of previous reports on GSH and MDA concentration in erythrocytes (Reglinski et al., 1988; Wang et al., 1999; Bryszewska et al., 1995). The protective effects of medicinal plant extracts on erythrocyte GSH and MDA concentration from hydrogen peroxide and *tert*-butyl hydroperoxide-induced oxidative stress may be attributed to the presence of polyphenols, tannins, anthocyanin, and glycosides which either have the capacity to scavenge free radicals or activate antioxidant enzymes or inhibit oxidases (Liu et al., 1992, 2006; Rice-Evans, 1995; Scartezzini & Speroni, 2000; Kulkarni et al., 2004; Amin & Hamza, 2005).

High polyunsaturated fatty acid, high cellular concentration of oxygen and hemoglobin in erythrocytes make them highly susceptible for oxidative damage which results in changes in membrane fluidity and inactivation of membrane-bound receptors and enzymes, ionic parameters (Maridonneau et al., 1983), an increase in lipid peroxidation (Rohn et al., 1998), oxidation of glutathione and protein sulphydryl group (Telci et al., 2000) and activation of proteolysis (Davies & Goldberg, 1987). Increased erythrocyte MDA concentration decreases the membrane fluidity of the lipid bilayer (Bryszewska et al., 1995) which causes the development of long-term complications in diseases like diabetes, hypertension, atherosclerosis, cardiovascular, cancer and neurological disorders (Halliwell & Gutteridge, 1990; Krouf et al., 2003; Siemianowicz et al., 2003).

Based on our observations, we hypothesize that use of medicinal plant extracts as a supplementary/dietary antioxidant in nutraceutical and/or cosmoceutical for protection against complications arising from oxidative stress may not only in part improve the ethnopharmacological knowledge of medicinal plants, but also pave the way for commercial utilization of medicinal plants. It seems worthwhile to mention that plants, which are more exposed to radical-forming radiation processes, are able to produce many types of scavenger molecules, mainly phenolic compounds. Mammals lack the ability to generate phenolic compounds (except estrogens), but this deficiency may be substituted for, in part, by the plants (Salah et al., 1995; Spiteller, 2003). Our observation may also, in part, explain the use of spent/waste part(s) of medicinal plants as an economically viable source of natural and potent antioxidant against complications arising from the oxidative stress.

Conclusion

The aqueous and alcohol extracts of *Punica granatum*, *Caesalpinia bonducella*, *Hibiscus subdariffa*, *Moringa oleifera*, *Garcinia indica,* and *Matricaria chamomilla* significantly protects the erythrocytes against oxidative stress induced by hydrogen peroxide and *tert*-butyl hydroperoxide. The extracts are capable of protecting erythrocytes against oxidative damage, thereby maintaining the basal levels of GSH and MDA concentration. The protection of erythrocytes GSH and MDA oxidation by medicinal plant extracts may be due to the presence of high amounts of polyphenolics, anthocyanin, tannins, glycosides to which its antioxidant activity may be ascribed. Further investigations on isolation, characterization and identification of active phytoceuticals responsible for the antioxidant activity are warranted for future work.

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