Age associated changes in erythrocyte membrane surface charge: Modulatory role of grape seed proanthocyanidins

Purushotham Sangeetha, Muthaiya Balu, Dayalan Haripriya, Chinnakannu Panneerselvam*

Department of Medical Biochemistry, Dr AL Mudaliar Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, India

Received 6 May 2005; received in revised form 21 July 2005; accepted 26 July 2005

Available online 31 August 2005

Abstract

Aging, a multifactorial process of enormous complexity is characterized by impairment of physio-chemical and biological aspects of cellular functions. It is closely associated with increased free radical production, which situation ultimately leads to devastation of normal cell function and membrane integrity. The present study was aimed to determine the effect of proanthocyanidins rich grape seed extract (GSP) on membrane surface charge density in erythrocytes during animal age associated oxidative stress. GSP (100 mg/day/kg body weight) was administered orally for 15 and 30 days to young and aged rats. Significant decrease in surface charge levels with concomitant increase in protein carbonyls and decrease in glycoprotein, antioxidants status was noted in erythrocytes of aged rats when compared with young rat erythrocytes. Duration dependent supplementation of GSP increased the erythrocyte surface charge density to near normalcy in aged rats. Decrease in protein carbonyls level and increase in glycoproteins as well as antioxidant status was observed in aged rat erythrocytes on GSP treatment. Thus, from our results, we conclude that GSP is an effective anti-aging drug in preventing the oxidative stress associated loss of membrane surface charge, which thereby maintains the erythrocyte membrane integrity and functions in elderly.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Aging; Erythrocyte surface charge; Protein carbonyls; Glycoprotein; Antioxidants; Proanthocyanidins

1. Introduction

Aging is the sum total changes that occur in living organisms with passage of time leading to functional impairment, increased pathology and death. It is a ubiquitous biological phenomenon associated with biochemical and functional alterations of the cells and tissues. The consequence for such decline in biological function in most of the cells and organ is attributed mainly to the age-dependent increase in production of free radicals (Harman, 1992). The exposure of free radicals on biological systems leads in many cases to increase the cellular levels of oxidatively modified proteins, lipids and nucleic acids, which coincidentally enhance the development of one or more well recognized, age related manifestations, including loss of physical activity, cellular functions and metabolic integrity. Reactive oxygen species (ROS) attack vital cellular protein component leading to the oxidation of amino acid side chains and protein backbones, formation of protein–protein cross linkages, inhibition of protein synthesis that ultimately results in protein fragmentation and increase of protein carbonyl levels, leading gradually to cell death (Bandyopadhyay et al., 1999).

Erythrocytes, the unique carriers of oxygen are highly susceptible to oxidative stress conditions. The rich polyunsaturated membrane lipids and iron, a potent catalyst for free radical reactions makes erythrocyte a good substrate for oxidative damages. Membrane oxidations do affect the intrinsic membrane properties as well, by altering membrane fluidity, ion transport and loss of enzymic activities of the cell (Chiu et al., 1989). As cell membrane is an important target for radical damage, and blood can reflect the liability of the whole animal to oxidative condition,
erythrocytes have been used extensively for determining the
effect of aging in studies concerning the possible involvement
of free radicals. The erythrocyte cell membrane has a
total negative electric charge, which determines the correct
course of many processes like transport of metabolic
substrates and products through ionic pumps, carriers and
membrane channels, for the transfer of information (Nalecz,
1989) and mainly to prevent aggregation of erythrocytes
from each other (Jovtchev et al., 2000). The aggregability of
erthrocytes is mainly determined by glycocalyx on their
membrane, in particular by the amount of the sialic acid
residues bearing negative surface charge. Oxidative stress or
other damaging effects to surface sialosaccharide may itself
play role in aggregation of erythrocytes, increasing the
adhesiveness to endothelial cells contributing for the
development of various pathologies including diabetes
mellitus, atherothrombotic complications (Wautier et al.,
1981) and sequestration of circulating erythrocytes by
macrophages (Beppu et al., 1995). In normal conditions, the
amount of oxy-repair enzymes or antioxidants would possibly
be sufficient to cope with the amount of damage
produced. However, during aging an oxidant challenge
exceeds the capacity of the cell’s defense system, membrane
damage may occur. During such conditions, dietary intake
of antioxidants gains immense importance.

Plant derived flavonoids are suggested to be natural
antioxidants associated with prevention of various
diseases and pathological conditions. There has been
increasing interest on grape seed flavonoids, as they are
rich sources of proanthocyanidin oligomers, the flavan-3-ols
that has number of biological and therapeutic
properties. Recently, grape seed extract have shown to
rejuvenate the antioxidant system in central nervous
system of aged rats (Balu et al., 2005), have demonstrated
cytotoxicity towards melanomas (Martinez Conesa et al.,
2005), has beneficial effect on bone formation and bone
strength for treating bone debilities (Yahara et al., 2005),
and to protect renal tissues against toxicity (Mohanasundari
et al., 2005). It also has antigenotoxic effect against
H₂O₂ in human lymphoblastoid cells (Sugisawa et al.,
2004), possess differential effects on insulin resistance,
hypertension, and cardiac hypertrophy (Al-Awwadi et al.,
2005) and even has favorable influence on vascular
function (Clifton, 2004) and anti-thrombotic effects
(Sano et al., 2005). Moreover, studies on erythrocytes
have reported that proanthocyanidins from cocoa have
strong inhibitory effect towards hydrogen peroxide,
xanthine oxidase, organic hydroperoxide, dialuric acid,
and 2,2′azobis(2 amidinopropane)dihydrochloride (AAPH)
induced hemolysis (Zhu et al., 2002). However, the effect
of GSP on erythrocyte membrane surface charges during
animal aging remains unexplored. Hence, the present
study was designed to examine the effect of GSP on
erthrocyte membrane surface charge, protein carbonyl
level and antioxidant status during animal aging.

2. Materials and methods

2.1. Chemicals

Dextran 500 (500,000 molecular weight), Polyethylene
glycol (PEG, 8000 molecular weight), Bovine serum albumin
(BSA), and 2,4-dinitro-phenylhydrazine (DNPH) were pur-
chased from Sigma Chemical and Company, Saint Louis, MO,
USA. All other chemicals were of reagent grade.

2.2. Animals and treatment

Male albino rats of Wistar strain, weighing approxi-
mately 120–160 g (young) and 380–420 g (old) were used.
The animals were obtained from King Institute of
Preventive Medicine, Chennai. The animals were divided
into two major groups namely, Group I: normal young rats
(3–4 months old) and Group II: normal aged rats (about 24
months old). Each group was further sub-divided into three
groups: one control group (Group Ia, IIa) and two
experimental groups based on the duration of GSP
administration for 15 days (Group Ib, Iib) and 30 days
(Group Ic, Iic). Each group consisted of six animals. The
animals were maintained on commercial rat feed containing
5% fat, 21% protein, 55% nitrogen free extract, 4% fiber
(w/w) with adequate mineral and vitamin contents and had
access to water ad libitum. GSP was supplemented orally at
the dosage of 100 mg/kg body weight/day for 15, 30 days,
whereas control young and aged rats received vehicle alone
in a similar manner.

On completion of 15, 30 days of GSP supplementation,
the blood was collected with 3.8% sodium citrate from
jugular vein and used for the isolation of erythrocytes and
erythrocyte membranes. The general physical health of rats
with respect to body weight and movement abilities were
monitored throughout the duration of GSP therapy and the
changes were found to be insignificant.

2.3. Preparation of proanthocyanidins rich grape seed
extract

Preparation of proanthocyanidins rich grape seed extract
was performed as described before (Zhao et al., 1999). The
total polyphenolic content of the extract obtained when
subjected to high performance liquid chromatography
contained 81.3% of proanthocyanidins, 6.7% of monomeric
catechins and 5.4% of organic acids.

2.4. Preparation of erythrocytes and erythrocyte
membranes

Isolation of erythrocytes and erythrocyte membranes was
done according to Dodge et al. (1963) with slight
modifications. Briefly, blood collected from animals with
3.8% sodium citrate was subjected to centrifugation at
3000 rpm for 10 min at 4 °C. The plasma and buffy coat
were removed by aspiration and the erythrocytes were washed three times with cold (4°C) phosphate-buffered saline (PBS: 0.15 M NaCl, 1.9 mM NaH2PO4, 8.1 mM Na2HPO4, pH 7.4). Washed erythrocytes were hemolyzed in 40 volumes of 5 mM sodium phosphate buffer (pH 8.0) (containing 1 mM EDTA and 0.5 mM phenylmethylsulfonyl fluoride, as protein inhibitor), and centrifuged for 20 min at 4°C at 15,000 rpm. The supernatant (or hemolysate) was decanted carefully and saved, while the pellet were washed repeatedly and incubated for 45 min at 37°C to reseal the membrane in the presence of 0.8 mM MgCl2-ATP solution, for hemoglobin free ghosts. Hemoglobin amount was estimated by Drabkin and Austin (1932) method and erythrocyte membrane protein content by Lowry et al. (1951).

2.5. Determination of erythrocyte surface charge

Erythrocyte partition coefficients were determined at room temperature by a two-phase aqueous system containing 5% Dextran 500 and 4.3% PEG 8000 in isotonic phosphate buffer (pH 6.8) by the method of Walter (1985). The two-phase system used is ‘charge sensitive’ whose partition coefficient ratio indicates the level of erythrocyte surface charge.

2.6. Determination of protein carbonyls

Protein carbonyls content was determined by the reliable method based on the reaction of carbonyl groups with 2,4-dinitro-phenylhydrazine to form 2,4-dinitro-phenylhydrazone as suggested by Levine et al. (1994).

2.7. Determination of glycoproteins

Lipids were extracted from the erythrocyte membrane pellets according to the method of Folch et al. (1957) using chloroform–methanol mixture (2:1 v/v). The resulting defatted residue was suspended in sodium acetate buffer (containing 2 mg cysteine HCl/ml, final pH 7.0) and deproteinized by 4–5 volumes of ethanol, evaporated to dryness in the cold under a vacuum and subjected to hydrolysis by heating with 2 ml of 4 N HCl for 4–6 h. The hydrolyzed material was neutralized with 4 N sodium hydroxide and used for estimating erythrocyte hexose (Niebes, 1972), hexosamine (Wagner, 1979) and sialic acid (Warren, 1959).

2.8. Determination of antioxidants

Superoxide dismutase (SOD) in hemolysate was assayed by the method of Marklund and Marklund (1974), while erythrocyte membranes were used to estimate catalase (CAT) (Beers and Seizer, 1952). Hemolysate glutathione peroxidase (GPx) was determined by Rotruck et al. (1973) method, glutathione reductase (GR) by the procedure of Staal et al. (1969), glucose-6-phosphate dehydrogenase (G6PD) by Zinkham et al. (1958) and glutathione-S-transferase (GST) according to Habig et al. (1974).

Hemolysate reduced glutathione (GSH) was estimated by Moron et al. (1979) and oxidized glutathione (GSSG) was assayed according to the method of Tietze (1969). Erythrocyte redox state was determined by the redox index: (GSH2/GSSG)/(2 × GSSG × 100).

Blood ascorbic acid was assayed according to Omaye et al. (1979) and erythrocyte membrane α-tocopherol content by the method of Desai (1984).

2.9. Statistical analysis

The results are expressed as mean ± standard deviation (SD) for six rats in each group. Differences between groups were assessed by one-way ANOVA using the SPSS version 7.5 software package for Windows, USA. Post hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test; statistical significance at p-values < 0.05, < 0.01, < 0.001 have been given respective symbols in the figures and tables.

3. Results

The young control (Group Ia) and aged control rats (Group IIa) received vehicle alone, whereas 15, 30 days treated young (Group Ib, Ic) and aged rats (Group IIb, IIc) received GSP orally at the dosage of 100 mg/kg body weight/day. The results obtained in erythrocytes of control

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Young rats</th>
<th>Aged rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ia (control)</td>
<td>Ib (15 days)</td>
</tr>
<tr>
<td></td>
<td>Ii (control)</td>
<td>Iib (15 days)</td>
</tr>
<tr>
<td>Partition coefficient ratio</td>
<td>0.82 ± 0.08</td>
<td>0.83 ± 0.08</td>
</tr>
<tr>
<td>Protein carbonyls (umoles DNPH/mg protein)</td>
<td>9.01 ± 1.08</td>
<td>8.9 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>0.48 ± 0.04 A*</td>
<td>0.62 ± 0.06 B#</td>
</tr>
<tr>
<td></td>
<td>14.38 ± 1.47 A*</td>
<td>11.55 ± 1.46 B*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats. Comparisons are between A-Group Ia with Ii, B-Group Iib, Iic with Group Ii, C-Group Iib with Group Iic. Values are statistically significant at *$p<0.05$, #*$p<0.01$, *$p<0.001$. 
and experimental young and aged rats are described in detail below.

Table 1 shows the age related changes in partition coefficient ratio and erythrocyte membrane protein carbonyls level. Normal partition coefficient ratio and normal protein carbonyls level were observed in erythrocytes of control and GSP treated young rats. These demonstrate membrane integrity and functions are maintained at normal levels in erythrocytes of young rat. However, significant decrease ($p<0.001$) in the partition coefficient ratio and increase in protein carbonyls level ($p<0.001$) were observed in aged rat erythrocytes when compared with young rat erythrocytes. The result illustrated significant surface charge loss and increased free radical attack on membrane proteins in aged animal. GSP administered aged rats significantly increased partition coefficient ratio on 15 days ($p<0.01$) and 30 days ($p<0.001$) treatment. Protein carbonyls level in aged rats was also decreased ($p<0.001$) on duration dependent GSP supplementation with significant difference between 15 (Group IIb) and 30 days (Group IIc) treatment at $p<0.05$.

Fig. 1 illustrates the levels of hexose, hexosamine and sialic acid in erythrocytes of control and GSP treated young and aged rats. The glycoprotein levels in erythrocytes of young rats were at normal level and did not show any significant alterations on GSP treatment. The aged control rats showed significant decrease in hexose, hexosamine and sialic acid by 17, 28, and 39%, respectively. GSP administration to aged rats for 15 days showed a significant increase in hexosamine ($p<0.05$) and sialic acid ($p<0.01$), while 30 days treatment showed increase of hexose at $p<0.01$, hexosamine at $p<0.001$ and sialic acid at $p<0.001$.

Table 2 indicates the erythrocyte enzymatic antioxidant status of control and GSP treated young and aged rats. Young control rats had normal level of enzymatic antioxidants and showed insignificant changes on GSP supplementation. However, significant decrease in the activities of SOD, CAT, GPx, GR, GST and G6PD at

Fig. 1. Levels of glycoproteins in erythrocytes of control and GSP treated young and aged rats. Control young (Group Ia) and aged rats (Group Ia) received vehicle alone. 15, 30 days treated young (Group Ib, Ic) and aged rats (Group IIb, IIc) received GSP orally at the dosage of 100 mg/kg body weight/day. Values are expressed as mean±SD for six rats. Comparisons are between A-Group Ia with IIa, B-Group IIb, IIc with Group Ia, C-Group IIb with Group IIc. Values are statistically significant at $p<0.05; \#p<0.01; *p<0.001; \text{ns, non-significant}$.

Table 2 Activities of enzymatic antioxidants in erythrocytes of control and GSP treated young and aged rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Young rats</th>
<th>Aged rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ia (control)</td>
<td>Ib (15 days)</td>
</tr>
<tr>
<td>SOD (U/mg Hb)</td>
<td>3.85±0.48</td>
<td>3.93±0.38</td>
</tr>
<tr>
<td>CAT (µmoles/min/mg protein)</td>
<td>4.15±0.58</td>
<td>4.21±0.54</td>
</tr>
<tr>
<td>GPx (U/g Hb)</td>
<td>7.03±0.75</td>
<td>7.06±0.77</td>
</tr>
<tr>
<td>GR (U/g Hb)</td>
<td>2.42±0.31</td>
<td>2.45±0.32</td>
</tr>
<tr>
<td>GST (µmoles of CDNB conjugated/min/mg Hb)</td>
<td>1.13±0.18</td>
<td>1.17±0.16</td>
</tr>
<tr>
<td>G6PD (U/g Hb)</td>
<td>6.00±0.55</td>
<td>6.05±0.60</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for six rats. Comparisons are between A-Group Ia with IIa, B-Group IIb, IIc with Group Ia, C-Group IIb with Group IIc. Values are statistically significant at $p<0.05; \#p<0.01; *p<0.001; \text{ns, non-significant}$.
p < 0.001 were observed in control aged rat erythrocytes when compared with young control rats erythrocytes. 15 days GSP administration to aged rats showed increase in the activities of SOD (p < 0.01), CAT (p < 0.05), GPx (p < 0.01), GR (p < 0.01), GST (p < 0.05) and G6PD (p < 0.001). While 30 days treatment demonstrated a significant increase in all the enzymatic antioxidant at p < 0.001 expect GST, which showed an increase at p < 0.01. Further, the difference between 15 and 30 days treatment for SOD, CAT, GPx, GR and G6PD was significant at p < 0.05. These results demonstrated that 30 days treatment is more efficacious than 15 days treatment in elevating the activities of decreased erythrocyte antioxidant status of aged rats to near normalcy.

Table 3 demonstrates the glutathione status in erythrocytes of control and experimental rats. Erythrocyte glutathione status was observed to be at normal levels in control and treated young rats. A significant decrease of GSH level with increase of GSSG levels in aged rats erythrocytes indicates the elevated GSH oxidation with animal aging. These alterations ultimately decreased the GSH/GSSG ratio and redox index (p < 0.001) in aged rat erythrocytes. However, increase of GSH content and decrease of GSSG levels with subsequent increase of GSH/GSSG ratio and redox index (p < 0.001) was observed on duration dependent GSP treatment, demonstrating the glutathione improving activity of proanthocyanidins.

Fig. 2 illustrates the blood ascorbic acid status and erythrocyte membrane α-tocopherol levels in control and experimental rats. GSP supplemented young rats showed insignificant changes in ascorbate and α-tocopherol levels when compared with young control rats. However, ascorbate and α-tocopherol levels were significantly decreased (p < 0.001) in erythrocytes of aged rats. Administration of GSP to aged rats increased the ascorbate and α-tocopherol levels on 15 days treatment at p < 0.01 and on 30 days treatment at p < 0.001. This indicates the favorable

![Fig. 2](image-url)
effect of GSP in improving the non-enzymatic antioxidant status as well in aged animals.

Correlation analysis study in aged control and treated rats showed, negative correlations between surface charge and protein carbonyls ($r = -0.678$, $p < 0.01$), GSSG levels ($r = -0.693$, $p < 0.01$). While positive correlations was observed between surface charge and activities of enzymatic antioxidants SOD ($r = 0.800$, $p < 0.001$), CAT ($r = 0.720$, $p < 0.001$), GPx ($r = 0.692$, $p < 0.01$), GR ($r = 0.662$, $p < 0.01$), GST ($r = 0.762$, $p < 0.001$), G6PD ($r = 0.891$, $p < 0.001$). Non-enzymatic antioxidants like ascorbic acid ($r = 0.795$, $p < 0.001$), α-tocopherol ($r = 0.650$, $p < 0.01$) and glutathione levels GSH ($r = 0.677$, $p < 0.01$), GSH/GSSG ($r = 0.892$, $p < 0.001$) also showed a positive correlation with surface charge levels. Glycoproteins like hexose ($r = 0.659$, $p < 0.01$), hexosamine ($r = 0.631$, $p < 0.01$) and sialic acids ($r = 0.761$, $p < 0.001$) when correlated with surface charge levels illustrated a positive correlation. Thus, except for protein carbonyls and GSSG levels all other parameter showed positive correlation with surface charge.

4. Discussion

Proper maintenance of membrane integrity and function is important for cell’s survival. The membrane interacts with both intra and extracellular environment that could bring about membrane changes probably responsible for the selective removal of erythrocytes prematurely from circulation. Conservation of the cell membrane structure and proper charge levels on its surface determines the correct course of many processes. As surface charge is a measure of cell condition, changes in surface charge indicate increased risk of erythrocytes to form aggregates contributing to many pathologic conditions. Even changes in erythrocyte structure and functions in chronic renal insufficiency and purulent intoxication were paralleled by changes in negative surface charges (Samoilov et al., 2002). A significant decrease of erythrocyte surface charge in aged rats when compared with erythrocytes of young rats was noted in our study. This decrease could be possibly due to the increased protein oxidation leading to carbonyl formations in aged rats erythrocytes. As previous results have shown, skeletal protein of erythrocytes when treated with oxidative reagent like diamide, hydrogen peroxide and phenylhydrazine results in cross linkages of sulfhydryl group and surface charge modifications (Fung et al., 1996). Earlier, our laboratory results demonstrated significant changes in the metabolism of protein in number of organs during oxidative stress associated aging in rats (Arivazhagan and Panneerselvam, 1999). Thus, increased oxidative damage on membrane proteins during animal aging does lead to the diminution of negative surface charge in erythrocytes.

Further, decrease of glycoproteins observed in aged rat erythrocytes is also thought to play a major role in surface charge determination. Studies have shown, both external and internal removal of glycoprotein, especially sialic acid on erythrocytes decreases surface charge level leading to the emergence of non-IgG covered epitopes on the surface of oxidized erythrocytes (Leb et al., 1987), consequently allowing the recognition of erythrocytes by macrophages (Beppu et al., 1995). Further, oxidation of glycoporphin A, major sialoglycoprotein decreases sialic acid content that eventually decrease membrane surface charges, contributing to rouleaux formation (Beppu et al., 1994). In addition, our results are in accordance with the work of Vomel (1984) who also reported that the decrease of sialic acid in old individuals results in surface charge modifications.

GSP supplementation showed insignificant changes in erythrocytes of young rats probably because they are not affected by oxidative stress conditions with cellular potential to resist to ROS. However, significant decrease of protein carbonyls and increase of glycoproteins level to near normalcy in erythrocytes of GSP treated aged rats would have ultimately increased the membrane surface charge density in them. These alterations were probably due to the free radical scavenging and reduced thiol group elevating properties of GSP (Sen et al., 1998) that protects the membrane proteins from oxidative insults. Previous reports have also proposed, flavonoids maintain the redox state of sulfhydryl residues in membrane proteins of HL-20 cells by oxidoreductase activity (VanDuijn et al., 1998).

Imbalance between radical production and catabolism of oxidant during aging would shift the cells towards oxidative stress resulting in alterations of membrane properties and cell dysfunction. Since, earlier studies have reported, stressed reticulocytes never mature to erythrocytes having normal membrane surface charges and gradually leads to the premature death of erythrocytes (Walter et al., 1975). SOD plays a key role in detoxifying superoxide anions, which otherwise damages the cell membranes and macromolecules. A significant decrease in SOD activity was observed in aged rats erythrocytes compared to young rats. This might be due to the increased superoxide radical formation via the Fenton reaction in aged animals. The increase of SOD activity in aged rat erythrocytes on GSP treatment may be due to the potential quenching of free radicals by proanthocyanidins through the formation of resonance-stabilized phenoxyl radical, which significantly decrease the superoxide radicals level (Rice-Evans et al., 1996). Other investigation by ESR techniques has also reported proanthocyanidins, as SOD equivalents in scavenging superoxide radicals very effectively (Virgili et al., 1998). Catalase, a hemoprotein that catalyzes the decomposition of H2O2, protects the cell from oxidative damage was decreased in our present study in erythrocytes of aged rats. Our data was analogous to prior reports, which showed decrease of erythrocyte CAT activities in old rats (Glass and Gershon, 1984). The reduction of CAT with animal aging could be because of the decrease in NADH, since NADH is necessary for the conversion of CAT from its inactive state.
GSP treatment increased CAT activity in aged rats via the increase of G6PD activity, which produces NADH by pentose pathway in erythrocytes.

GPx efficiently protects the cell membrane from lipid peroxidation and catalyzes the reaction of hydroperoxides with GSH to form GSSG. As glutathione is a reducing agent because of its sulphhydryl groups against oxidative stress, depletion of glutathione causes a proportional decrease of GPx activity (Spector et al., 1993). Thus, the decreased GSH in erythrocytes of aged rats could be the reason for the inhibited activities of GPx and GST, since these enzymes act only at the expense of GSH. Further, decreased G6PD activity with decrease of GR in aged rat erythrocytes shows the inability of the system to maintain GSH/GSSG ratio. Liu and Choi (2000) has also reported rat erythrocyte GSH levels are lower in 24 months old rats when compared to 3 month old rats. Moreover, earlier studies have shown decrease in GR activities and GSH content with animal age (Stio et al., 1994). These reasons could possibly explain for the altered GSH/GSSG and redox index in erythrocytes of aged rats. However, GSP treatment increased the GSH level that ultimately increased the GPx, GST GR, G6PD levels to near normalcy in aged rats. The reason was probably due to the protection of sulphhydryl groups in glutathione from oxidative damages via free radical quenching action of di-OH (catechol) structure in the B ring of proanthocyanidin in GSP (Ishige et al., 2001).

The decrease of ascorbic acid in aged rats compared to young rat erythrocytes was because of the increased oxidative stress with animal aging. The decrease of ascorbate ultimately damages the erythrocyte membrane, since they are involved in regeneration of ω-tocopherols, the lipophilic membrane antioxidant from its oxidized form. Our results showed that GSP were capable of reversing the decline of vitamin C in the aged rats. This would have been possibly due to the (i) increased ascorbic acid absorption, (ii) stabilization of ascorbic acid, (iii) reduction of dehydroascorbate to ascorbate, and (iv) metabolic sparing of ascorbic acid by the flavonoids (Hughes and Wilson, 1977) in GSP. In addition, based on the former information of Yamasaki and Grace (1998), proanthocyanidin prolong ascorbate radical lifetime by one-electron reduction of dehydroascorbic acid and regenerate ascorbic acid from its corresponding radical. Further, the increased oxidisability of fatty acids in aged rat erythrocytes decreases vitamin E, as they protect the cellular bilayer from free radical attack. Elevation of vitamin E levels in GSP administered aged rats was probably through the chain breaking action of proanthocyanidins within the core of phospholipids bilayer, which save vitamin E and recycle them from the tocopheroxyl radical through an H-transferring mechanism, thereby behaving as a sacrificial antioxidant (Maffei Facino et al., 1998). Moreover, the metal chelating property of GSP increase vitamin E content by preventing the involvement of hemoglobin iron in lipid peroxidation processes (Maffei Facino et al., 1996). Taken together, GSP potentially increase the enzymatic and non-enzymatic antioxidants levels in aged rat erythrocytes. These anti-oxidants protect erythrocyte membrane from free radical attack eventually preventing the loss of surface charge levels during age associated oxidative stress.

In summary, we found that erythrocyte membrane surface charge is decreased in aged rats possibly due to the increased protein oxidation and decreased enzymatic and non-enzymatic antioxidant levels with advancement of animal age. GSP treatment significantly decreased protein oxidation and increased antioxidant status that concomitantly increased the surface charge density in erythrocytes of aged rats in duration dependent manner. The outcome of the present study thus indicated, GSP supplementation could be a possible therapeutic measure and an exact remedy for minimizing the age related alterations in erythrocytes for the existing population, as the health care and welfare of the aged have been attracting considerably as a social problem. Thereby, GSP acts as a potent anti-aging drug for preventing health disorders and for improving the well being of elderly to lead a normal life span free of ailments.

Acknowledgements

The first author, P. Sangeetha gratefully acknowledges the financial assistance provided in the form of Junior Research Fellowship (JRF) awarded by Indian Council of Medical Research (ICNR), New Delhi, India.

References


