

# Age associated erythrocyte membrane damages in rats: potential role of chloroform extract of *Solanum trilobatum*

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The current surge of attention to improve the quality of life of an organism is nowadays paying attention on natural therapies. Thus, this particular research is focused to study the age associated erythrocyte membrane damages in rats and the protective effect of the antioxidant rich Chloroform extract of *Solanum trilobatum*. The erythrocyte membrane damages and the protective role of Chloroform extract of *Solanum trilobatum* (150mg/kg body weight orally for 4 weeks) in aged rats was assessed by the levels of lipid per oxidation, lipid profiles, membrane fluidity, membrane bound enzymes and the antioxidant status as an incidence of oxidative stress on comparing with the young rats. A significant ( $p<0.05$ ) increase in the lipid peroxidation levels with a significant alteration in lipid profiles, membrane fluidity and a decrease ( $p<0.05$ ) in membrane bound enzyme activities in aged rat erythrocytes was restored ( $p<0.05$ ) on treatment with Chloroform extract of *Solanum trilobatum*. The results of this study showed that chloroform extract of *Solanum trilobatum* on long-term administration in erythrocytes of rats showed immense membrane stabilizing effect with advancement of age for which the phenolic content of the studied plant material could be a good indicator of antioxidant capacity. With the development in techniques and recent researches, it has been proved that flavonoids which were earlier thought to be of no importance to human diet possess antioxidant properties.

**Keywords:** Antioxidant, Phenolic compounds, Chloroform extract of *Solanum trilobatum*, Membrane fluidity, Membrane bound enzymes, Glutathione reducing enzymes, Lipid preoxidation.

## INTRODUCTION

The imbalance between cellular production of Reactive Oxygen Species (ROS) and the ability of cells to defend themselves against the free radicals is referred to as *Oxidative stress*. Oxidative damage apparently increases with age and thus may overwhelm the natural repair system in the organism (Kowald, 1999) leading to the onset of age-related diseases with a progressive increase in the chance of morbidity and mortality. At the cellular level, the ROS inclination due to oxidative stress damages vital cell components like poly unsaturated fatty acids (PUFA), protein and nucleic acids (Bandyopadhyay

et al., 1999). This further enhances the development of one or better-recognized age related modifications, such as alteration of intrinsic membrane properties (fluidity, ion transport, loss of enzyme activity, protein crosslinking), inhibition of protein synthesis and DNA damage (Stadtman et al., 1992), ultimately resulting in the cell death.

Erythrocyte is an ideal model for studies related to ageing as they are devoid of a nucleus and other intracellular organelles, thus cannot yield direct information on the DNA control of cellular ageing, and do allow the age related modifications of intracellular proteins and plasma membrane to continue without the interference of the repair processes and exchange of damaged macromolecules (Bartos, 1998). Changes in

the macromolecules of membrane are one of the earliest signs of erythrocyte membrane alterations during ageing. ROS generated in the aqueous or lipid phase can attach to the erythrocyte membrane and can induce oxidation of lipids, proteins and carbohydrates, triggering disruption in membrane (Grune et al., 2001). Oxidation of lipids or *Lipid Peroxidation* is characteristically a free radical chain reaction initiated by the abstraction of a hydrogen atom within the cells due to high concentration of PUFA in the RBC membrane (Bennett, 1990). Studies have shown membrane lipid alterations, as an important factor in decreasing lipid fluidity during ageing in erythrocytes resulting in erythrocytes membrane rigidity (Borst et al., 2000). The rigidity of membrane seems to be an important factor in reducing cell deformability, the phenomenon due to enhanced intracellular viscosity (Borst et al., 2000). Alterations in membrane fluidity during ageing thereby lead to disturbance in blood rheological behaviour contributing to various pathophysiological implications including cardiovascular diseases, hypertension and stroke (Szapary et al., 2004).

The free radical theory of aging thus serves and plays a prominent role in invoking antioxidants as powerful defense mechanisms protecting the cells and tissues from oxidative stress as an anti-ageing movement. However erythrocytes are prone to have shown cumulative oxidant mediated damages due to endogenously and exogenously produced radicals with advancement of age. Antioxidant vitamins Vitamin E and vitamin C may prevent or mitigate lipid peroxidation. Vitamin E (alpha-tocopherol) is considered the predominant lipid-soluble, chainbreaking micronutrient antioxidant. In vitro studies suggest that vitamin C may enhance the effects of vitamin E by reducing tocopheroxyl radicals (Meagher et al., 2001). In some animal studies, a high intake of vitamin C increased tissue vitamin E concentrations (Afnas, 2005). Deficiency of vitamin E may cause neurological dysfunction, myopathies and diminished erythrocyte life span (Kawamoto et al., 2005). The molecular mechanism of these functions is believed to be mediated by either the antioxidant action of the vitamin or by its action as a membrane stabilizer (Ward et al., 2005).

Epidemiological data suggest that dietary antioxidants may have a beneficial effect on many age-related diseases. The beneficial impact of antioxidants on various age-related degenerative diseases may forecast an improvement in life span and enhance quality of life. The intake of plant derived natural antioxidants is recently more in practice, as the usage of synthetic antioxidants has long been questioned for its safety. *Solanum trilobatum* is one such medicinal herb rich in antioxidant supplement with wide beneficial biological property. *Solanum trilobatum* extract consists of compounds including solasodine, linoleic acid, palmitic acid, oleic acid, stearic acid (Saradha Vasanth, 1990), sobatum, □-

sitosterol, disogenin and β-solamarine responsible for many biological properties (Mohan and Devi, 1998). Recently a strong antioxidant activity has been reported in the chloroform extracts of the plant *Solanum trilobatum* due to the presence of phytochemicals such as iso flavonoids, phenols, phenolic acids, xanthenes and lignans (Sini and Devi, 2004; Amir and Kumar, 2005). *Solanum trilobatum* possessed a broad spectrum of antibacterial, antifungal and antitumoural activities of cough (Govindan et al., 1999). The decoction of various parts of the plant is used in chronic bronchitis. The berries and flowers of *Solanum trilobatum*, are widely used to treat respiratory diseases in Indian traditional system of Medicine (Kirtikar and Basu, 1993). Recently Mohan and Rathinam (1997) has also proved that *Solanum trilobatum* possess free radical scavenging activity when exposed to the ultraviolet rays. Reports have shown that active principle from *Solanum trilobatum* possessed antitumor effect against chemically induced tumors (Govindan et al., 1999; Shahjahan et al., 2005). Thus this particular research was aimed to evaluate the age-related oxidative stress in erythrocyte membrane of young and aged rats and the protective effect of the antioxidant rich Chloroform extract of *Solanum trilobatum* (CST).

## MATERIALS AND METHODS

### Preparation of chloroform extraction of *Solanum trilobatum*

The leaves of *Solanum trilobatum* were collected from the local market and samples of the plant were identified and authenticated by Dr. Brindha, Botanist, Department of Pharmacognosy, and Captain Srinivasa Murti Drug Research Institute for Ayurveda (CCRAS, New Delhi), Arumbakkam, Chennai, and Tamil Nadu. The fresh leaves (700g) were shade dried, powdered and extracted successively with 1.2 L of chloroform, in a Soxhlet extractor for 18-20 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40°-50°C). The chloroform, extract yielded a brown semisolid residue weighing 6.2 g (2.39% w/w). The extract was then filtered through Whatmann No.1 filter paper and concentrated using concentrator. Then it was frozen and subjected to lophilization.

### Animals

Male albino wistar Young (3-4 months, 120-150 g) and aged (22-24 months, 380-410 g) rats were used for the experiments. The rats were housed in polypropylene cages on a 12L: 12D cycle and fed *ad libitum* on commercial laboratory food pellets and water. All animal experiments were conducted as per the instructions of Institutional Animal Ethics Committee

### Experimental Design

The animals were divided into four major groups of six animals namely, Group I: Control young rats were received sterile water only, Group Ib: Young rats were administered with CST for 90 days, Group II: Control aged rats were received sterile water only, Group

IIb: Aged rats were administered with CST for 90 days.

### Dosage fixation

Various doses of Chloroform extract of *Solanum trilobatum* (50 mg, 100 mg, 200 mg, 300 mg and 400 mg/kg body weight) were used once daily for 4 weeks in 22-24 months aged Wistar rats (380-410 g) to assess the effective dose of the extract and duration of treatment against aging based on the contents of erythrocyte lipid peroxidation (LPO). Pretreatment with Chloroform extract of *Solanum trilobatum* at doses of 200 mg, 300 mg and 400 mg/kg body weight for 4 weeks were found to be effective in aged rats. The minimal effective dose 150 mg/kg body weight/day of dose was fixed as therapeutic dosage for the subsequent studies.

### Preparation of erythrocytes and erythrocyte membrane

The erythrocyte membrane was isolated according to the method of Dodge et al. (1963) with slight modification. 4 ml of packed cells was washed thrice with Tris-HCl buffer (310 mM, pH 7.4). Hemolysis was then performed by pipeting out the washed erythrocyte suspension into polypropylene centrifuge tubes that contained 5 mM sodium phosphate buffer, pH 8.0 and left for 1 hr. Erythrocyte ghosts were sedimented by centrifuging for 40 min at 30,000-x g. The supernatant (or hemolysate) was decanted carefully and saved, while the pellet was washed repeatedly. The colorless framework of erythrocyte remaining after hemolysis is referred to a 'ghost' or 'stroma' or 'stromata' or 'post-hemolytic residues'. The membrane ghost was then immediately re-suspended in 0.1 M Tris-HCl buffer, pH 7.4 and frozen, stored by submerging the tubes with the membrane suspensions in liquid nitrogen at  $-80^{\circ}\text{C}$  until further assay.

### Determination of fluorescent anisotropy

Lipid fluidity of erythrocytes was measured by fluorescence anisotropy technique, using fluorescent lipid probe 1, 6 diphenyl hexa 3, 4, 5 triene (DPH), according to the method of Shinitzky and Barenholz (1974). A small volume of DPH solution (2 mM) in tetrahydrofuran (THF) was injected with rapid suspension and was stirred for at least 2 hr after which no odour of THF was detected. The erythrocytes incubated in PBS were mixed with DPH suspension to give a final probe concentration of 1n mol/ml and the mixture was incubated at  $37^{\circ}\text{C}$  for 15 min. Fluorescence polarization was measured at  $25^{\circ}\text{C}$  in Perkin Spectrofluorimeter equipped with polarizers using an excitation wavelength of 360 nm and emission wavelength of 420 nm with a period of exposure of sample to the excitation light less than 10 sec. The steady-state fluorescence anisotropy (rDPH) was calculated according to Van Blitterswijk (1981).

### Biochemical estimation

#### Erythrocyte Membrane Bound Lipids

#### Extraction

Total lipids were extracted from the membrane by the method of Folch (1957) using Chloroform: Methanol (2:1 v/v) mixture. The membrane pellet obtained from 0.5 mL of packed cells was treated with 7.0 mL of methanol and homogenized in a Poltter-Elvehjem homogenizer. The contents were filtered and the residue on the

filter paper was carefully allowed to evaporate below  $60^{\circ}\text{C}$ . The lipid extract was redissolved in a known volume of chloroform - methanol mixture (2:1 v/v). To this was added 2.0 mL of 0.1 M potassium chloride. The contents were shaken well and centrifuged. The aqueous phase was washed again with potassium chloride: methanol: Chloroform taken in the ratio of 10: 10:1 (v/v), 3 times and each time the upper was discarded. Aliquots of the final lipid extract (chloroform layer) were used for lipid assay. Total cholesterol level in erythrocyte membrane and plasma were estimated by the method of Parekh and Jung (1970). Total phospholipids in erythrocyte membrane and plasma were estimated by the method of Rouser (1970).

### Membrane bound assays

Lipid peroxidation in erythrocyte membrane was assayed according to the method of Beuge and Aust, (1978) with slight modification.  $\text{Na}^+ \text{K}^+$  ATPase was assayed according to the method of Bonting, (1970).  $\text{Ca}^{2+}$  ATPase was estimated according to the method of Hjerten and Pan (1983).  $\text{Mg}^{2+}$  ATPase was assayed by the method of Ohinishi et al, (1982). Inorganic phosphorus was estimated by the method of Fiske and Subbarow, (1925).

### Assay of antioxidants

Total sulphhydryl group was estimated according to the method of Sedlack and Lindsay (1968). Ascorbic acid was estimated by the method of Omaye et al. (1979). The level of  $\alpha$ -tocopherol was estimated by the method of Desai (1984).

### Statistical Analysis

Values are mean  $\pm$  SD for six rats in each group and significance of the differences between mean values were determined by one-way analysis of variance (ANOVA) followed by the Duncan test for multiple comparison.

## RESULTS

Figure 1 illustrates the level of lipid peroxidation, blood ascorbic acid and  $\alpha$ -tocopherol in control and CST treated young and aged rats. Levels of malonaldehyde (MDA) were profoundly increased by 1.7 fold in erythrocytes and 1.8 fold in plasma of aged rats when compared to young rats. Administration of CST significantly reduced the level of lipid peroxidation in erythrocyte membrane by 1.3 fold and in plasma by 1.5 fold respectively, thereby displaying the antioxidant capacity of CST in combating oxidative stress in aged animals. Significant decrease in the levels ascorbic acid by 35% and  $\alpha$ -tocopherol by 30% was observed in aged rats when compared to young rats. CST supplementation to aged rats significantly increased ascorbic acid (32%) and  $\alpha$ -tocopherol (30%) levels, demonstrating the non-enzymatic antioxidant elevating property of CST.

Table 1 shows the level of fluorescence anisotropy, the levels of cholesterol and phospholipids and the cholesterol / phospholipid (C/P) ratio in erythrocyte

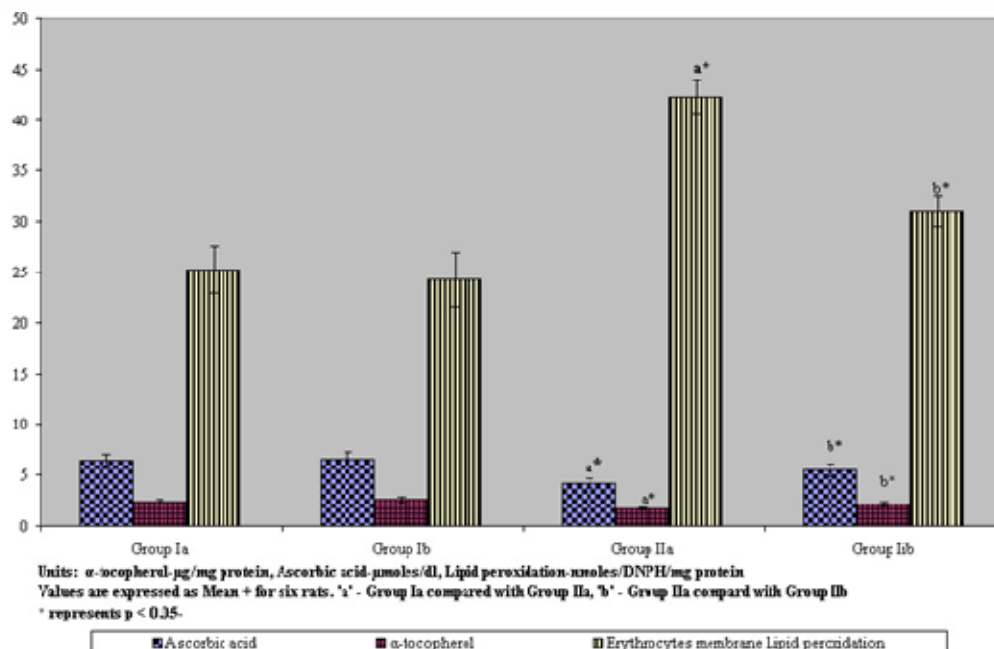
**Table 1.** Total Cholesterol, Total Phospholipid Cholesterol: Phospholipid Ratio and Fluorescence Anisotropy in Erythrocyte Membrane of Control and CST Treated Young and Aged Rats

Parameters	Young rats Group Ia	Group Ib	Aged rats Group IIa	Group IIb
Cholesterol	120.52 ± 12.24	115.12 ± 11.28	181.32 ± 17.01 <sup>d</sup>	135.26 ± 14.23 <sup>u</sup>
Phospholipid	241.59 ± 23.72	246.40 ± 21.02	173.16 ± 15.12 <sup>a*</sup>	228.24 ± 20.71 <sup>b*</sup>
C/P ratio	0.53 ± 0.05	0.51 ± 0.04	1.21 ± 0.09 <sup>a*</sup>	0.59 ± 0.05 <sup>b*</sup>
DPH	0.29 ± 0.02	0.27 ± 0.03	0.38 ± 0.04 <sup>a*</sup>	0.33 ± 0.03 <sup>b*</sup>

'a' - Group IIa compared with Group Ia, 'b' - Group IIb compared with Group IIa \* represents  $p < 0.05$

Units: cholesterol, phospholipid -  $\mu\text{g}/\text{mg}$  protein ;

Values are expressed as Mean  $\pm$  SD for six rat

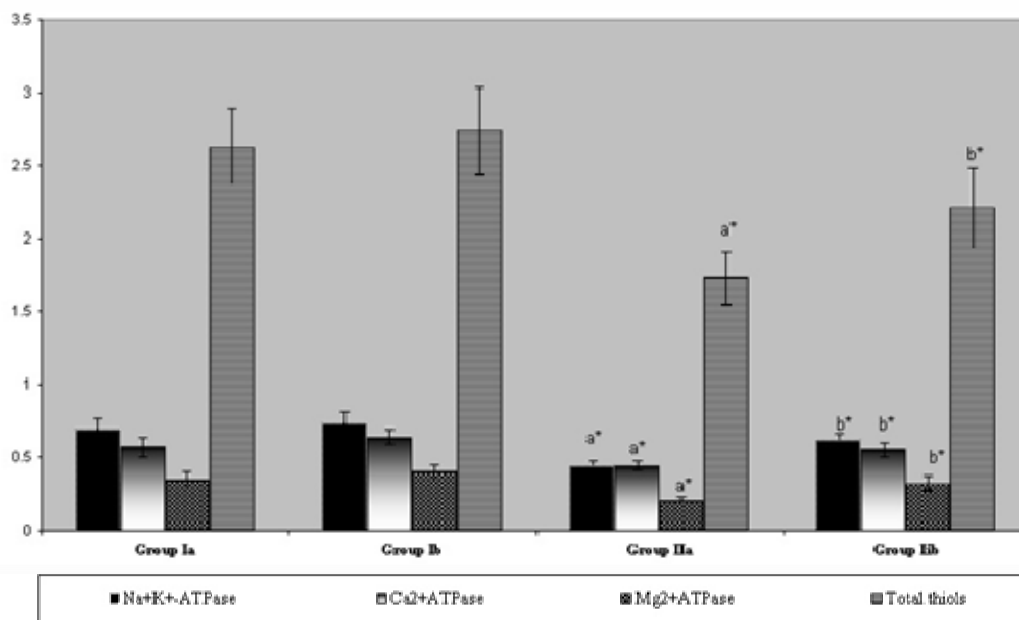
**FIGURE 1 LEVELS OF ANTIOXIDANTS AND LIPID PEROXIDATION IN ERYTHROCYTE MEMBRANE OF CONTROL AND CST TREATED YOUNG AND AGED RATS**

membrane of control and CST treated young and aged rats. Fluidity of membrane is important in maintaining the structure and function of erythrocytes. Erythrocyte membrane fluidity was measured in terms of fluorescence anisotropy using fluorescent lipid probe, 1,6 diphenyl hexa 3,4,5 triene (DPH). Fluorescence anisotropy was significantly increased by 31% in erythrocytes of aged rats when compared with young rats illustrating an increase in erythrocyte membrane fluidity with advancement of age. CST treatment to aged rats significantly increased the level of anisotropy by 13% probably through the membrane protective and antioxidant role of flavonoids present in CST. The levels of cholesterol were elevated by 50% and phospholipids

were declined by 28% significantly in erythrocyte membrane of aged rats when compared with young rats. CST treatment to aged rats significantly decreased the levels cholesterol by 25% and increased phospholipids by 31% in erythrocyte membrane. Significant increase ( $p < 0.05$ ) in the C/P ratio was observed in aged rats ( $p < 0.05$ ) when compared to young control rats. CST supplementation to aged rats decreased the cholesterol levels and increased phospholipid levels thereby restoring the C/P ratio.

Figure 2 represents the activities of ATPase and the levels of total thiol in the erythrocyte membrane of control and CST treated young and aged rats. Significant decline in the activities of  $\text{Na}^+\text{K}^+\text{ATPase}$  by 36%,  $\text{Ca}^{2+}\text{ATPase}$

**Figure 2 : ACTIVITIES OF MEMBRANE-BOUND ENZYMES AND TOTAL THIOLS IN CONTROL AND CST TREATED AGED AND YOUNG RATS**



Units : Na<sup>+</sup>K<sup>+</sup>-ATPase, Ca<sup>2+</sup>-ATPase, Mg<sup>2+</sup>-ATPase -  $\mu$ moles of p<sub>i</sub> liberated/min/mg protein and Total thiols- $\mu$ moles/dl  
Values are expressed as Mean  $\pm$  for six rats. 'a' - Group Ia compared with Group IIa, 'b' - Group IIa compared with Group IIb

by 56% and Mg<sup>2+</sup>ATPases by 51% were observed in erythrocytes of aged rats. CST administration to aged rats improved the activities of these ion motive ATPase by 32% for Na<sup>+</sup>K<sup>+</sup>ATPase, 21% for Ca<sup>2+</sup>ATPase and 58% for Mg<sup>2+</sup>ATPases. This confirms the protective effect of CST in maintaining the ionic gradient in erythrocytes with advancement of animal age. Significant decrease in the levels of total thiols by 34% was noticed in erythrocytes of aged rats compared to young rats. CST supplementation to aged rats increased the total thiol levels in erythrocytes by 20%.

## DISCUSSION

Several lines of evidence have been used to support the free radical theory including the claims that: enhanced expression of antioxidative enzymes in experimental animals can produce a significant increase in longevity and cellular levels of free radical damage increases with age (Fusco et al., 2007). Since the ageing process is characterized as time dependent, the age related modification of lipid structures should be considered as one of the major contributors for the age dependent diseases (Pepe et al., 1997). The high polyunsaturated fatty acid content of erythrocyte membrane and the continuous exposure to high concentration of oxygen and iron in hemoglobin are the factors that make erythrocytes very sensitive to the lipid peroxidative injury (Hulbert et

al., 2007). The end products of LPO, namely malonaldehyde (MDA) are a highly reactive bifunctional molecule and have been shown to cross link erythrocyte phospholipids and proteins to impair variety of the membrane related functions and ultimately to diminished RBC survival (Kumar et al., 2009). Consequently such alterations in lipids have been suggested to be the cause of ageing and age-associated degenerations (Spiteller, 2003; Spiteller, 2002). Significant increase in the levels of MDA in erythrocyte membrane of aged rats indicated the possibility of increased radical production, and higher rate of LPO in the present study. Our results are in agreement with that of other authors who has reported the increase in concentrations of LPO products including MDA in erythrocytes of rats (Gurer et al., 1997), man (Marotta et al., 2007) and rabbits (Brzezi ska-Slebodzińska, 2007). Administration of CST brought down the oxidative stress substantially and decreased the MDA levels in the erythrocyte membranes of aged rats. Flavonoids (isoflavonoids) present in CST scavenge lipidperoxides by binding metal ions and by inhibition of enzymatic systems responsible for free radical generation (such as 5-lipoxygenase, cyclo-oxygenase, and mono-oxygenase or xanthine oxidase) thereby declining LPO levels in aged rats (Cotelle et al., 1996).

The age related membrane rigidity and impaired membrane-related functions diminished RBC survival which may probably be due to the increase in lipid peroxide levels formed upon oxidation of iron released

from hemoglobin that could in turn cause alteration in erythrocyte antioxidant defense systems (Yanagawa et al., 1999). Thus decrease in the antioxidants such as vitamin E and vitamin C in erythrocytes of aged rats, could enhance the lipid peroxidation process in the present study. Therapeutic supplementation of CST enhanced significantly the levels of ascorbic acid and  $\alpha$ -tocopherol in aged rats to near normalcy. The increase in the level of vitamin C would have been possibly due to the increased absorption and stabilization of ascorbic acid, reduction of dehydroascorbate to ascorbate, and metabolic sparing effect of ascorbic acid by the flavonoids (Hughes and Wilson, 1977) in CST. As an antioxidant,  $\alpha$ -tocopherol intercalates into phospholipid bilayers with the long axis of the molecule oriented parallel to the lipid hydrocarbon chains and prevents the propagation of free radical reactions, (Vannucchi et al., 2003; Ward et al., 2005). Elevation of vitamin E levels corresponds to the action of polyphenols that regenerate  $\alpha$ -tocopherol from tocopherol radical through an H-transferring mechanism which thereby behaves as a sacrificial antioxidant (Carini et al., 2000). Moreover, the metal chelating property of CST increase vitamin E content by preventing the involvement of hemoglobin iron in lipid peroxidation processes (Maffei Facino et al., 1996).

Cell membrane fluidity at an optimum state is necessary for the right functioning of the membrane (Zicha et al., 1999). Various gerontological studies have illustrated altered membrane fluidity with advancement of animal age (Yu et al., 1992). In the present study, DPH probe was used to monitor the fluidity changes in erythrocyte membranes. Their fluorescence anisotropy values respond to lipid arrangement in various regions of membranes. Significant increase in fluorescence anisotropy therefore indicated the increased membrane rigidity in aged rat erythrocytes in the present study. A significant increase in lipid hydroperoxide content and a marked decrease in the fluidity of the erythrocyte membrane in old rats can be attributed to our study (Takasaki et al., 2002). Cholesterol plays a key role in erythrocyte membrane fluidity as it appears to be the main bilayer matrix in an intermediate fluid that regulates the mobility of phospholipid fatty acyl chains by condensing hydrophobic interaction leading to increased rigidity to membrane lipids. Reports by Marino et al., 2002 recognized an increase in cholesterol with the advancement of age may be the underlying cause for the membrane rigidity. In accordance our experimental results, demonstrated a significantly higher susceptibility to peroxidation, and a significant increase in the cholesterol and decrease in phospholipids contents with a concomitant increase in the ratio between cholesterol and phospholipids of the erythrocyte membranes of aged rats in comparison with the young rats. Parallel studies evidenced a significant decrease in erythrocyte phospholipids (Lopez -Revuelta et al., 2005) and

augmented cholesterol levels during aging (Harman, 2003). Reduction in total amount of phospholipids in erythrocyte membrane, the composition of its subclasses and the asymmetrical distribution across the membrane bilayers may be owed to cause instability and dysfunction of the membrane (Schroeder, 1984).

Supplementation of CST to the aged rats increased the membrane fluidity to near normalcy indicating the protection afforded by the active components (saponins, tannins, anthocyanin, betacyanin, isoflavonoids, phenols, phenolic acids, xanthenes and lignins) present in the CST. Phytochemicals present in *solanum trilobatum* can potentially reduce oxidative modifications of membrane by restraining the access of oxidants to the bilayer and propagation of lipid oxidation in the hydrophobic membrane matrix thereby improving their fluidity (Halder and Bhaduri, 1998). The polyphenols effectively lowers the total cholesterol and LDL oxidability level by inhibiting cholesteryl ester hydroperoxides formation (Preuss et al., 2000; Vinson et al., 2002) thereby supporting the decreased levels of cholesterol levels in CST treated aged rats. Further the decrease in erythrocyte membrane rigidity on CST may possibly due to the hypolipidemic role and ROS scavenging effect of flavanoids (quercetin) present in CST (Sudheesh et al., 1992). CST was found to be effective in increasing the phospholipid levels, due to the property of flavanoids which are known to anchor the polar head of main phospholipids through hydrogen bonds forming reversible physiochemical complexes (Sendra et al., 2007; Roychowdhury et al., 2001).

Erythrocyte membrane bound ATPases play an important role in the maintenance of the ionic gradients between the intracellular and extra cellular compartment of the cell. Changes in the ionic concentration can bring about diverse ripple of cell injury and ultimately cell death (Trump et al., 1993). Significant decrease in the activities of  $\text{Na}^+\text{K}^+$  ATPase,  $\text{Ca}^{2+}$  ATPases and  $\text{Mg}^{2+}$  ATPases was observed in the erythrocytes of aged rats compared to young rats. It is evident that modification in the fatty acid composition of red cell phospholipids change the allosteric behavior of membrane bound enzymes (Yu et al., 1992). The most possible mechanism for the alterations in ATPases activity is through changes in lipid-protein interactions due to the amendment of the lipid-bilayer environment caused by free radicals and LPO during the transport of ions in ageing (Tsakiris et al., 2006). As thiol status also contributes in maintaining the structure and function of ATPases (Liu and Wei, 1999), the decrease in thiol levels in erythrocytes of aged rats as evidenced from the present study could also be a possible reason for the decrease in ATPases activity in aged rats erythrocyte. The phenolic compounds present in CST, (Zainol et al., 2003) with different functional properties such as scavenging of ROS, LPO chain breaking activity and thiol group replenishing properties (Laranjinha et al., 1995) accounts for the improvement of ATPases in aged rats. Further, membrane stabilizing

effects and the  $\text{Ca}^{2+}$  chelating property of phenolic compounds of CST (Pawlikowska-Pawlega et al., 2000) might also contribute for the enhanced activity of ATPases in aged rats.

Thus the identification of free radical reactions as promoters of the aging process implies that interventions aimed at limiting or inhibiting them should be able to reduce the rate of formation of aging changes with a consequent reduction of the aging rate and disease pathogenesis. In recent times the search for natural antioxidants and other preparation of plant origin to slow, stop or reverse ageing has been intensified to promote beneficial effects in aged animals. Plant-derived polyphenols are significant constituent of the human diets and their free radical scavenging capabilities protect erythrocytes against oxidative insult during ageing. The antioxidant and membrane protective role of *Solanum trilobatum* provide an efficient way to alleviate the macromolecular damages and protect from premature cell death with advancement of age. In expense of these studies, *Solanum trilobatum* is suggested as a novel life prolonging paradigm in laboratory animals. Therapy of flavonoid rich extract may not necessarily increase mean life span but they may extend the quality of life. Ultimately the study will help in designing elderly care which will lead to increase mean life expectancy.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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