# Altered Redox Status in Erythrocytes From Hypertensive Subjects: Effect of (−)Epicatechin

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**Abstract** Hypertension is a major problem worldwide. There is much evidence to suggest that reactive oxygen species (ROS) radical may play a role in the development of organ damage associated with cardiovascular disease and hypertension. (−)Epicatechin, a member of tea catechins belonging to flavonoid group, is known to be a potent anti-oxidant. The study has been undertaken to evaluate the effect of (−)epicatechin on markers of oxidative stress: reduced glutathione (GSH) and membrane sulfhydryl (—SH) groups in erythrocytes from hypertensive patients. The effect of (−)epicatechin was also compared with a known anti-oxidant L-ascorbic acid. The erythrocyte intracellular GSH content and membrane —SH group content were significantly ($P$<0.01) decreased in hypertensive subjects. *In vitro* incubation with (−)epicatechin caused an increase in GSH and —SH content, the effect was more pronounced in hypertensive erythrocytes. Similar results were obtained with L-ascorbic acid. The observed decrease in the level of GSH and —SH groups in hypertension is an indicator of oxidative stress condition. Observation of an increase in red cell GSH content and the protection of membrane —SH group oxidation by (−)epicatechin in hypertensive subjects is a convincing reason to suggest that high dietary intake of foods rich in catechins may help to reduce oxidative stress and concomitant free radical damage in hypertensive patients.

**Key words** human, erythrocytes, hypertension, (−)epicatechin, oxidative stress

Hypertension is a major health problem worldwide. It is the main risk factor for heart attack, heart failure and stroke. Hypertension affects 10% ~20% of the population in developing countries and is recognized as an important risk factor for the development of various disease. It has been estimated that in 2001, 17 million people died of cardiovascular diseases (CVD) of all type.

Oxidative stress is thought to play a role in the etiology of large number of degenerative diseases including cardiovascular, cancer and neurological disorders [1,2]. There is much evidence to suggest that reactive oxygen species (ROS) radical may play a role in the development of organ damage associated with CVD and hypertension. Oxidative stress has also been shown to play an important part in the pathogenesis of atherosclerosis [3]. It is hypothesized that high blood pressure is associated with loss of balance between peroxidation and anti-oxidant factor in the body[4].

Recently, much attention has been focused on the anti-oxidant properties of flavonoids, a large class of polyphenolic compounds derived from plants. Evidence suggest that these compounds may protect tissue against damage caused by oxygen free radicals and lipid peroxidation [5]. Observational studies have suggested an inverse relationship between flavonoid intake and risk of cardiovascular disease in humans[6,7].

(−)Epicatechin, a member of tea catechins belonging to flavonoid group, is known to be a potent anti-oxidant. Earlier we have reported the anti-oxidant effect of (−)epicatechin on erythrocytes from type 2 diabetic patients [8]. The present study has been undertaken to evaluate the effect of (−)epicatechin on markers of oxidative stress: reduced glutathione (GSH) and membrane sulfhydryl (—SH) groups in erythrocytes from hypertensive patients. We also compared the effect of (−) epicatechin with a known anti-oxidant L- ascorbic acid.

## 1 Materials and methods

### 1.1 Selection of subjects

Venous blood from 26 hypertensive subjects (18 male and 8 female) were taken after informed consent. The mean age of hypertensive subjects was (53.3±5.4) years, mean systolic pressure was (150.6±6.7) mmHg and diastolic pressure was (109.9±4.8) mmHg. The BMI of the subjects ranged between 25.5 kg/m$^2$ to 29.5 kg/m$^2$.

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The control group was gender and age matched with hypertensive subjects. The mean systolic blood pressure was (118.6±4.3) mmHg and diastolic blood pressure (79.3±4.1) mmHg. The BMI of control group ranged between 22 kg/m$^2$ to 24 kg/m$^2$. All experiments were carried out with in 3~4 hours of blood collection.

All chemicals were of highest purity available. (−)Epicatechin was procured from Sigma Chemical Company, St. Louis, USA. Other chemicals were purchased from Loba Chemie or Himedia, India.

### 1.2 Measurement of erythrocyte intracellular GSH

The blood sample was centrifuged for 10 min at 100 *g* to remove plasma. The isolated erythrocytes were washed 3~4 times with 0.154 mol/L NaCl at 4℃ to remove leucocytes. Intracellular GSH was measured following the method of Beutler[9]. Concentration of GSH is expressed in micro moles per gram of hemoglobin (μmol·L$^{-1}$·g$^{-1}$).

### 1.3 Measurement of erythrocyte membrane —SH group

Washed erythrocytes were used to isolate plasma membrane following the method of Marchesi and Palade[10]. The erythrocyte membrane —SH group was estimated by the method of Kitajima *et al*.[11].

Both the methods of GSH and —SH group estimation are based on the ability of sulfhydryl groups to reduce 5,5- dithiobis- 2- nitrobenzoic acid (DTNB) and generate a yellow coloured anionic product whose optical density is measured at 412 nm. A molar extinction coefficient of 13 600 was used for the nitro benzoate ion. Concentration of —SH group is expressed in term of nanomoles per mg of protein (nmol·mg$^{-1}$). Hemoglobin was estimated by ferricyanide/cyanide method of Beutler[12]. Protein was estimated by Lowry's method[13].

### 1.4 *In vitro* incubation with (−) epicatechin/ L-ascorbic acid

*In vitro* experiments were carried out by adding (−)epicatechin/L-ascorbic acid to blood and incubating at 37℃ for 60 min. The protocol was similar as previously reported in the case of diabetic erythrocytes[14]. In parallel control experiments blood was incubated without (−)epicatechin or L- ascorbic acid. Experiments were also conducted to determine whether the presence of (−)epicatechin or L-ascorbic acid affected the DTNB assay method. (−)Epicatechin or L-ascorbic acid did not interfere with DTNB assay method.

## 2 Results

The erythrocyte intracellular GSH content and membrane —SH group content were significantly ($P$<0.01) decreased in hypertensive subjects. *In vitro* incubation with L- ascorbic acid caused an increase in both GSH and —SH content of erythrocytes, however, the effect was more pronounced in hypertensive erythrocytes (Figure 1a and 2a). Decreasing the concentration of L-ascorbic acid gave less effect and no significant effect was observed at $10^{-7}$ mol/L and lower concentration.

Under similar experimental conditions, incubation with (−)epicatechin also resulted in increase in erythrocyte GSH and —SH content in both control and hypertensive subjects (Figure 1b and 2b). The effect of (−)epicatechin was more pronounced in hypertensive erythrocytes. The effect of (−)epicatechin was very similar to the one observed with L-ascorbic acid.

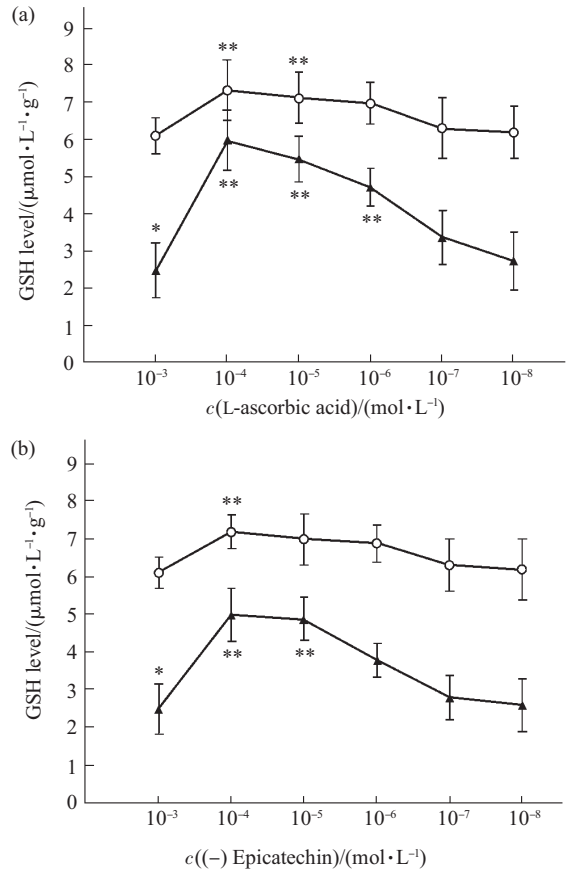


**Fig.1 Concentration dependent effect of L-ascorbic acid (a) and (−) epicatechin (b) on intracellular reduced glutathione in normal and hypertensive erythrocytes**

$\bar{x}\pm s$, $n$=5,6. *$P$ < 0.01 compared with normal, **$P$ < 0.01 compared with respective untreated controls. ○—○: normal, ▲—▲ : hypertensive.

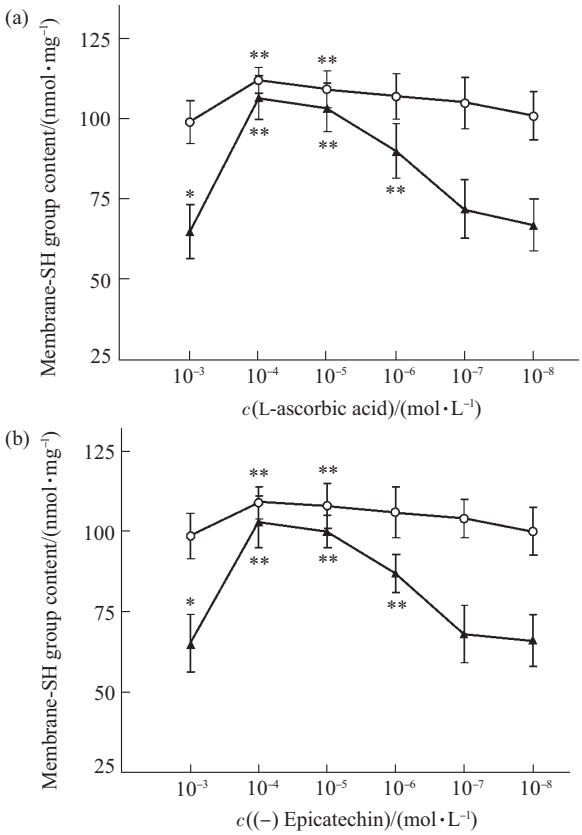


**Fig.2 Concentration dependent effect of L-ascorbic acid (a) and (−) epicatechin (b) on membrane —SH group level in normal and hypertensive erythrocytes**

$\bar{x}\pm s$, $n$=5,6. *$P$ < 0.01 compared with normal, **$P$ < 0.01 compared with respective untreated controls. ○—○: normal, ▲—▲ : hypertensive.

## 3 Discussion

Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and the hydroxyl radical are known to play a role in organ damage associated with hypertension. Hypertensive patients exhibit a significantly higher production of ROS than normotensive subjects [15]. Even among normotensive subjects, those with a family history of hypertension have a higher production of plasma peroxide than normotensive with no family history of hypertension. Thus there seems to be a genetic relation between hypertension and oxidative stress [16]. The total anti-oxidant status and total plasma anti-oxidant capability is decreased in hypertensive subjects[4].

Erythrocytes have been extensively used to study alteration during hypertension. Changes in membrane fluidity[17], anti-oxidant enzyme activity[4], ion transport and membrane lipid composition [18,19], intracellular potassium content[20] have been reported. Alteration in erythrocytes have been used as markers to study pathogenesis of hypertension[21]. Our observation of a decreased red blood cell GSH content in hypertensive subjects is corroborated by other reports [22,23]. Membrane —SH group is also a marker of oxidative stress since its level is dependent on total anti-oxidant status of the plasma. A decreased —SH content in hypertensive erythrocytes, as observed in the present study, is a further proof of oxidative stress in hypertension.

Epicatechin is a member of a group of polyphenolic compounds collectively known as catechins, present predominantly in green tea. Epicatechin has also been isolated and identified to be the active principle present in the bark of the tree *Pterocarpus marsupium*. The aqueous extract from the bark of *Pterocarpus marsupium* has been used for centuries as an anti-diabetic drug in Indian folk medicine, scientific research has shown that epicatechin has certain insulin-like properties [23]. Recent studies have shown that catechins have many important biological properties: protection against cancer [24], vasorelaxant effect [25], hypoglycemic action [26], modulation of platelets function [27] and anti-allergic action [28]. The present study provide further evidence for the strong anti-oxidant effect of (−)epicatechin, an effect that is almost similar to that of L-ascorbic acid, in erythrocytes from hypertensive patients. In the past there have been some doubts over the bioavailability of dietary flavonoids, however recent reports conclusively show that flavonoids are absorbed from the small intestine [29]. A direct relationship is observed between consumption of polyphenol rich diet and plasma concentrations of flavonoids [30]. Intake of tea catechins affects the concentration of endogenous anti-oxidants in the plasma and has the potential to maintain anti-oxidant activity [31], this effect contributes to prevent cardiovascular diseases[32].

The red cell is exposed to higher oxygen tension than other cells and is more susceptible to oxidative stress. The membrane —SH groups play an important role in the maintenance of membrane structure and function of red cells. As part of their anti-oxidant defense, erythrocytes contain high level of GSH to prevent oxidation of hemoglobin —SH groups, and of the intra-membranous and cytoskeletal proteins. The observed decrease in the level of GSH and —SH groups in hypertension is an indicator of oxidative stress condition. Our observation of an increase in red cell GSH content and the protection of membrane —SH group oxidation by (−)epicatechin in hypertensive subjects is a convincing reason to suggest that high dietary intake of foods rich in catechins may help to reduce oxidative stress and concomitant free radical damage in hypertensive patients.

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