INTRODUCTION

Red blood cells (RBCs) are one of the most susceptible biological tissues to oxidative stress due to the presence of both high concentration of polyunsaturated fatty acids (PUFA) in the membrane and the oxygen transport associated with redox active hemoglobin molecules, which are promoters of reactive oxygen species (ROS). Lipid peroxidation involves the cleavage of unsaturated fatty acids at their double bonds producing short-chained aldehydes. The occurrence of lipid peroxidation causes alteration in RBC membrane structure, function and alteration of membrane bound receptors and enzymes.

Phenylhydrazine (PHZ) is one of the most investigated intracellular free radical generating probes which promote oxidative damage in erythrocytes. Treatment of normal RBCs with PHZ, produces features that are characteristic of RBC phenotypes in severe β-thalassaemia, namely, rigid and mechanically unstable membranes in conjunction with selective association of oxidized α-globin chains with the membrane skeleton. As such, PHZ was suggested to serve as an in-vitro model for beta-thalassemic RBC phenotypes. During β thalassemia this type of condition is created due to the iron overload and hampers the antioxidant system of the RBC membrane. Trace metals, especially iron are implicated as causative agents in excessive generation of free radical which are capable of causing oxidative damage to erythrocytes. PHZ in the presence of hemoglobin autooxizes to form both superoxide and hydrogen peroxide radicals, which ultimately give rise to hydroxyl radical, each with the capacity to initiate peroxidation of unsaturated fatty acids in endogenous phospholipids. Iron is probably released from the denatured hemoglobin, which
may promote the conversion of superoxide anion and hydrogen peroxide into the very reactive hydroxyl radical through the Haber Weiss reaction.

Since there are no mitochondria in erythrocytes, these cells depend on less efficient pathways for production of high-energy compounds, the anaerobic glycolytic (Embden-Meyerhof) pathway, which is also known as the hexose monophosphate shunt or the phosphogluconate pathway. Under normal circumstances, about 90% of glucose entering the red cell is metabolized by the anaerobic pathway and 10% by the aerobic pathway. It has been reported that glucose 6-phosphate dehydrogenase (G6PDH) and other metabolic enzymes like hexokinase and aldolase activity is reduced and lactate dehydrogenase (LDH) activity is increased during oxidative stress.

Membrane-bound enzymes are important in maintaining the normal physiology of erythrocytes. Free radicals can induce degenerative changes in erythrocytes which disturbs the structural integrity of the membrane that might in turn affect the activities of membrane bound enzymes like Na\(^+\)/K\(^+\)-ATPase, Mg\(^{2+}\)-ATPase and Ca\(^{2+}\)-ATPase and acetylcholinesterase (AchE) activity. Level of nitric oxide (NO), is another oxidative stress marker which also increased during stress condition. NO is responsible for the conversion of oxyhemoglobin to methemoglobin (MHb). Increased NO status also indicates the increased nitric oxide synthase (NOS) activity in the cell.

*Terminalia arjuna* (TA) (Combretaceae family) a deciduous and evergreen tree, standing 20-30m in height is found in abundance throughout Indo-sub-Himalayan tracts of Uttar Pradesh, South Bihar, Madhya Pradesh, Delhi and Deccan region near ponds and rivers. Among different species of Terminalia, the bark of TA has its own characteristic
features. The active constituents of TA include tannins, triterpenoid, saponins (arjunolic acid, arjunic acid, arjungenium, arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), oxalic acid, ellagic acid, gallic acid, oligomeric proanthocyanidins (OPCs), phytosterols, polyphenols, calcium, magnesium, zinc and copper. TA is also an important medicinal plant widely used in the preparation of Ayurvedic formulations for over three centuries primarily as a cardiac tonic in India. Clinical evaluation of this plant indicates that it can be of benefit in the treatment of coronary artery diseases, heart failure and possibly hypercholesterolemia. It has also been found to be antibacterial and antimutagenic. But, most of the beneficial works on this plant have been carried out on the alcoholic extract of its bark. However, its aqueous bark extract also showed novel protection mechanism in several in vitro systems like RBC, liver tissue, and heart mitochondria and its antioxidant mechanisms were also established. The significance of using this aqueous extract of TA bark for our studies lies in the fact that, it is the form in which it is consumed directly by the tribals and rural people of India as medicines for their various health problems.

Hence, our present study was aimed at exploring whether this aqueous bark extract of TA, is capable of protecting the cytoskeletal architecture of the RBC membranes, the membrane bound enzymes and the enzymes of metabolic pathway of an aerobic oxidation in RBCs from phenylhydrazine induced oxidative stress in vitro and whether antioxidant mechanisms are associated with such protection.