SUMMARY
Background

Polyphenols are the most abundant antioxidants in the diet and are widespread constituents of fruits, vegetables, cereals, dry legumes, chocolate, and beverages, such as tea, coffee, or wine. Experimental studies on animals or cultured human cell lines support a role of polyphenols in the prevention of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, and osteoporosis. As antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated with oxidative stress.

*Allium* is the largest and most important representative genus of the Alliaceae family and comprises 450 species, widely distributed in the northern hemisphere. Besides the well known garlic (*Allium sativum* L.) and onion (*Allium cepa* L.), several other species are widely grown for culinary use, such as leek (*Allium porrum* L.), scallion (*Allium fistulosum* L.), shallot (*Allium ascalonicum*), wild garlic (*Allium ursinum* L.), elephant garlic (*Allium ampeloprasum* L. var. *ampeloprasum*), chive (*Allium schoenoprasum* L.), Chinese chive (*Allium tuberosum* L.). Since ancient times garlic and onion have been used as common foods and for in the form of traditional medicine for the treatment of many diseases. They are among the oldest of all cultivated plants with their origin in central Asia. The first citation of these plants is found in the Codex Ebers (1550 BC), an Egyptian medical papyrus reporting several therapeutic formulations based on garlic and onions as useful remedy for a variety of diseases such as heart problems, headache, bites, worms and tumours.

Onion (*Allium cepa* L.) is one of the most important vegetables worldwide with an estimated annual production of almost 64 million tons in the year 2008. Onion (*Allium cepa* L.) is of great economic importance, and is the second most important vegetable crop in the world. India being a second major onion producing country in the world has a productivity of 10.16 MT/ha. According to the Food and Agriculture Organization (FAO) onions are grown in at least 175 countries. Besides making a significant nutritional contribution to the human diet, onions also have medicinal and functional properties. Onion has been recognized as an important source of valuable phytonutrients as flavonoids, especially flavonols with a wide range of quercetin,
isorhamnetin and kaempferol conjugates, fructo-oligosaccharides, saponins, thiosulfinates and other sulphur compounds.

Onion has been described to have several health benefits related to its antioxidant, anticarcinogenic, hypolipidemic, hypoglycaemic, or antiaggregatory effects. Many epidemiological studies suggest that regular consumption of onions in food is associated with a reduced risk of neurodegenerative disorders, many forms of cancer, cataract formation, ulcer development, reduction in symptoms associated with osteoporosis, prevention of vascular and heart diseases by inhibition of lipid peroxidation (LPO) and lowering of low density lipoprotein (LDL) cholesterol. Their biologically active molecules are effective antioxidants against the lethal effect of oxidative stress. They are also reported to have liver protective effect, immune enhancement potential and anti-infection, anti-stress, anti-cancer and other pharmacological properties.

At least 25 different flavonols have been characterised in onions. Onions ranked highest in quercetin content in a study of 28 vegetables and 9 fruits. Quercetin concentration in onions ranges from trace amount in white to 2.5–3mmol / kg in red varieties in which it occurs as various O-glycosides with d-glucose as the main sugar residue. Quercetin in onions exists in four predominant forms: quercetin aglycone, quercetin-3,4-diO-glucoside, querceti-3-O-glucoside and quercetin-4-O-glucoside. Distribution of quercetin and its glycosides within the onion bulb changes during onion processing, different cooking methods and exposure to fluorescent light.

Quercetin shows a variety of pharmacological effects such as growth inhibition of tumour and microbial cells, reduction of cancer risk, scavenging of free radicals, protection against cardiovascular disease, anti-ageing and rejuvenating effects. Quercetin is known to exert multiple mechanisms including antioxidant activity, anti-inflammation, modification of signal transduction pathways, and interactions with receptors and other proteins. Previous studies have shown that quercetin could ameliorate diabetes-induced oxidative stress and preserved pancreatic beta cell. It could enhance adiponectin secretion by a PPAR-c independent mechanism, prevent impairment of insulin sensitivity without affecting body weight and, inhibit hepatic
stellate cells, activation and reduce the expression of human C-reactive protein and cardiovascular risk factors (such as undergoing apoptosis and fibrinogen) in mice.

Erythrocytes are exposed to high oxidant stress may result in accelerated peroxidation reactions and cellular aberration. Protective mechanisms exist to scavenge and detoxify ROS, block production, or sequester transition metals. Erythrocytes are well equipped with several biological mechanisms to defend against intracellular oxidative stress comprising antioxidant system consists of enzymatic and nonenzymatic pathways. Enzymes for preventing oxidative denaturation in erythrocytes include SOD, CAT, GSHPx, GSH reductase-dependent regeneration of GSH, and NADH–metHgb reductase. Endogenous non-enzymatic antioxidants are defined in two phases: lipophylic (vitamin E, carotenoids, ubiquinon, melatonin, etc.) and water soluble (vitamin C, glutathione, uric acid, ceruloplasmin, transferrin, haptoglobin, etc.). Several exogenous compounds such as inhibitors of NADPH Oxidase, allopurinol, and flavonoids have antioxidant properties. It is known that flavanoids are good exogenous antioxidants against free radical initiated lipid peroxidation in human red cells and that the antioxidant activity of flavanoids depends significantly on molecular structure and initiation conditions.

In recent years, there is renewed interest towards study of plants and their isolated compounds for the prevention of diseases and diverse pathological conditions by offering protection against cellular damage and oxidative stress. Researches on traditional medicinal plants are gradually emerging as viable alternatives to conventional drugs for various free radical mediated diseases. Onion, one of the oldest cultivated plants used for culinary, medicinal and spiritual purposes, recently gaining attention for its multiple functional properties. The phytochemical composition of onions is believed to vary according to species and cultivation technique. Some researchers reported that the red onion is abundant in polyphenols, flavonoids, flavonol, and tannin and had quercetin levels that were 14-fold that of garlic and levels that were two-fold that of white onions. In a study of the antioxidant effects of onion flesh and peel, rats that consumed the peel had higher levels of antioxidants and lower levels of lipid peroxide than rats that ate the flesh did. Previous studies show significant differences in the levels of QDG and QMG between different onion
cultivars, with the outer dry peel having greater capacity for controlling lipid peroxidation than the flesh does.

Taking these results together, research on their biochemical and protective effect *in vitro* and *in vivo* to evaluate the differences in the levels of antioxidants between different layers of the edible part of the onion is warranted. The objective of the present study focuses on the study of antioxidant properties during different stages of development of onion and also in different layers of the edible part of the onion. The study was undertaken to understand better the most suitable part of the onion for the extraction of flavonoids with regard to human nutrition. The present study also focuses on the antioxidant effect of onion extract, on biomarkers of oxidative stress in erythrocytes, plasma and tissues (brain and liver) in a rat model of experimental oxidative stress. Their effects were compared with quercetin (abundant in onion itself) and catechin (abundant in tea) taken as standard.

**Results Achieved and Major Findings**

**Antioxidant Activity and Proximate Composition of Different Layers of Onion Extract at Two Different Stages of Maturation In vitro**

In the first section of the study, onion extract showed significant antioxidant activity *in vitro* with outer living layers exhibiting more phenolic content and flavonoid content and showing variable distribution of flavonoids (quercetin) in different scales. Total phenolic content (TPC) was evaluated, as a function of 1) size of onion (big and small), 2) in inner layers and outer layers. Outer living layers had higher contents of TPC (ranging from 84.4 to 97.8 mg) followed by a continuous decrease towards the inner part of the bulb (ranging from 48.6 to 52.5 mg). Difference was also observed between TPC of outer layers, being higher in smaller onion (p < 0.05). A higher flavonoid content of outer living layers (ranging from 40.27 to 45.0 mg) followed by a continuous decrease towards the inner part of the bulb (ranging from 29.10 to 31.81 mg) were also reported. Difference was also observed between flavonoid content of outer layers, being higher in smaller onion (p < 0.05).
The antioxidant activity (AOA) of onion extracts was estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe-(II) complex. Large differences in FRAP values were observed among different layers and sizes of onions, ranging 34.33 to 56.4 in case of outer layers and 14.21 to 16.24 µM Fe²⁺/g fresh weight in case of inner layers, showing high AOA in outer layers of onion with greater AOA of smaller onion (p < 0.01). However, variation in AOA in different layers of onion might be due to variation in the quantities of quercetin in the various layers of onion extracts showing that antioxidant activity in onions is positively correlated with total flavonoid contents with respect to different layers. All the onion extracts were studied for their FRSA using DPPH* radical and compared with quercetin of different concentrations (10⁻⁵ mol/L and 10⁻⁶ mol/L). The FRSA of the extract showed a wide variation in % DPPH inhibition ranging from 17.28 to 32.33 %. Outermost living layers of the onion extracts were found most powerful free radical scavenger compared to the inner layers with higher FRSA of outer layers of smaller onion.

Ability of the onion extracts to scavenge hydrogen peroxide was also determined. Higher scavenging activity of outermost living layer (ranging from 43.0% to 56.33%) when compared to inner layers (ranging from 28.39% to 29.4%) were reported. Higher scavenging activity of standard molecule, quercetin was also reported (67.19%). At 50 µg/mL concentration of all extracts, the outermost living layer extract showed higher reducing power (absorbance ranging from 0.22 to 0.30) than inner layer extract (absorbance ranging from 0.073 to 0.081). Quercetin, reference compound, exhibited the high reducing activity of 0.408 in 10 µg/mL concentration. Outermost living layers of the onion extracts were found most powerful hydroxyl radical scavenger compared to the inner layers with higher FRSA of outer layers of smaller onion. Quercetin also shows a high HRSA activity at different concentrations. The ferrous chelating abilities of onion extracts were compared with that of quercetin and EDTA. The values demonstrates that EDTA which serves as the positive control showed the highest percentage of the chelating effect and the chelation rate reached 90.71 ± 3.76% at 100 µg/mL. The chelating ability of the different onion extracts were 34-35.6% in case of outer layers and 21.6-28.5 % in case of inner layers at 100 µg/mL concentration.
In the present study, inhibitory activity of different sizes and layers of onion against porcine pancreatic α-amylase were studied and compared with quercetin. Results showed that in porcine pancreatic α-amylase, quercetin were potent inhibitors with inhibitory activity ranging from 69.6 to 84.3% at different concentrations respectively. Differences in % inhibitory activity were also observed among different layers and sizes of onions, ranging 52 to 44.8 % in case of outer layers and 36 to 39.12 % in case of inner layers, showing high inhibitory activity in outer layers of onion (p < 0.05).

The outer living layers exhibit higher free radical scavenging activity, reducing capacity, metal chelating activity and hydroxyl radical scavenging activity; statistical analysis also showed these activities of outer layers to be significantly correlated with total phenolic content of outer layers (p < 0.05). The difference in phenolic content and antioxidant activity of outer layers with respect to different sizes of onion could be explained on the basis of their lesser moisture content correlated to the higher phenolic content. Also, outer layers of smaller onion showed higher scavenging activities than outer layers of larger onion with no significant difference with quercetin (taken as standard), this observation further provides evidence for difference in phenolic content and antioxidant activity in different stages of development and in different layers of onion.

**Effect of Different Layers of Onion Extract on Oxidative Stress Biomarkers in Erythrocytes subjected to Oxidative Stress by tBHP In vitro: Comparison with Quercetin**

Many *in vitro* and *in vivo* studies have demonstrated that several parameters of erythrocyte function and integrity are negatively affected by increased oxidative stress. Because of their high susceptibility to oxidation, erythrocytes have been used as a metabolically simplified model system to investigate oxidative damage in biomembranes. The present study demonstrates *in vitro* protective effect of onion extract on lipid peroxidation, reduced glutathione (GSH), erythrocyte hemolysis, plasma membrane redox status (PMRS) activity with respect to different layers (the outermost living layers just beneath the dry outer scales of onion and the inner layers),
in an effort to categorize the antioxidant efficacy in different parts of the onion (p < 0.01).

**Effect of Onion Extract on Oxidative Stress Biomarkers in Erythrocytes subjected to Oxidative Stress by Mercuric Chloride *In vivo*: Comparison with Quercetin and Catechin**

Onions have been found to be effective in the prevention and treatment of a number of diseases and have antidiabetic, anti-biotic effects, cardioprotective effects, anti-cancer, and anti-infectious properties, therefore, research on the physiological effects of red onion on disease prevention *in vivo* is warranted. Our study demonstrates that aqueous extracts of onion may provide protection against HgCl₂ induced oxidative damage by possibly reducing lipid peroxidation and increasing the antioxidant defence mechanism in rats (p < 0.01).

Results showed that onion extract maintained protein redox status by mitigating various protein oxidation products (PCO, AOPP and P-OOH levels) (p < 0.05) and plasma sialic acid levels since structural changes in proteins are considered to be among the molecular mechanisms leading to endothelial dysfunction and development of many chronic diseases including aging.

PON1 functions as an antioxidative enzyme that inhibits the oxidation of LDL or protecting the endothelium from the pro-oxidant effect of oxidized LDL and its direct role in reducing oxidative stress. Supplementation of onion extract (p< 0.05) and quercetin (p< 0.001) to oxidatively stressed rats significantly improved the PON 1 levels as compared to HgCl₂ group. An increase in susceptibility of LDL was observed in HgCl₂ treated oxidatively stressed rats (Pearson’s r = 0.9970, p< 0.001), however, supplementation of onion extract, quercetin and catechin significantly (p< 0.05) decreased the plasma oxLDL levels in oxidatively stressed rats as compared to HgCl₂ group. Onion extract up regulates and preserves PON1 activity and reduces LDL oxidation, our results provide evidence of the positive effect of onion extract on PON1 activity and prevention of LDL oxidation during periods of oxidative insult, with improvement in the antioxidant capability of rats, suggesting its value as a
dietary antioxidant food. The findings may explain the anti-atherosclerotic effect of onion and also foods containing quercetin and catechins.

The PMRS is increasingly recognized as a major mechanism for reducing plasma membrane associated oxidative stress and as a protective mechanism that operates to maintain the ascorbate level in plasma which is crucial for maintaining the redox balance. Onion extract protects the erythocytes in vivo from its oxidation induced damage through its strong antioxidant activity by normalizing erythrocyte PMRS activity, thus, maintaining a redox state in the plasma by reducing extracellular oxidants (p < 0.01). The polyphenols (flavonoids mainly quercetin) present in onion exert their antioxidant activity by their ability to enter the erythrocytes and donate electrons to PMRS. This property is a compensatory/protective mechanism that operates to maintain the ascorbate level in plasma and thereby minimize oxidative stress.

**Effect of Onion Extract on Oxidative Stress Biomarkers in Brain and Liver Tissues subjected to Oxidative Stress by Mercuric Chloride In vivo: Comparison with Quercetin and Catechin**

Tissue and physiological dysfunction occurs as the oxidative stress increases. Our study demonstrates a protective effect against lipid peroxidation (MDA) and protein oxidation (PCO content) in tissues (brain and liver) against oxidative stressed rats. The results also revealed their positive effect on enzymatic (CAT, SOD and Gpx) and non enzymatic (GSH) antioxidants and on plasma membrane redox system in tissues (p < 0.01).

**Effect of Different Layers of Onion Extract on Oxidative Stress Biomarkers in Alloxan Induced Diabetic Rats In vivo: Comparison with Quercetin and Insulin**

The protective effect of different layers of onion on oxidative stress biomarkers in alloxan induced diabetic rats was investigated in comparison with quercetin. Administration of onion extracts (OLE and ILE) to diabetic rats caused marked hypoglycemic activity by progressive reduction in the blood glucose levels in alloxan-induced diabetic rat model which indicates antidiabetic potentials of the extract. The results revealed a decreased serum TG, TC, LDL-c and VLDL-c levels.
Supplementation of onion extracts: outer layer extract (OLE) and inner layer extract (ILE), caused marked reduction in lipid peroxidation and increased GSH content of erythrocytes, antioxidative activity of plasma and decreased plasma sialic acid level shown in this study is the novel finding as an indicative of a decrease progression of diabetic complications (p < 0.01). OLE showed higher antioxidant activity and GSH content as compared to ILE (p < 0.05).

**Conclusion**

The present study demonstrates difference in antioxidant properties during different stages of development of onion and also in different layers of the edible part of the onion. In conclusion, it can be stated that the outer living layer (the transitional layer with the first living cells below the dry onion peel) is a better resource for food ingredients and easily accessible source for nutraceutical compounds.

Since the inhibition of α-amylase has been suggested as a strategy for diabetes control, our study demonstrates a higher inhibitory activity of outer layers of onion against porcine pancreatic α-amylase as compared to inner layers. These findings are correlated with strong lowering effect of outer layers against biomarkers of oxidative stress in diabetic rats *in vivo* as compared to inner layers, stating that this fraction of onions has a strong anti diabetic effect. This promising result encourages further investigations including bioassay of the fractionated extract which may lead to the isolation of compound(s) that are responsible for the hypoglycemic effects and which can be further developed to modern anti-diabetic drugs.

Given the anti-oxidant properties of onion and quercetin and the link between ageing and oxidative stress, the quercetin–mediated protection of PON1 levels by onion extract may also be a putative anti aging strategy. The findings may explain the anti-atherosclerotic effect of onion and also foods containing quercetin and catechins. The present study thus provides enough evidence for the strong biological antioxidant activity of the onion extracts against biomarkers of oxidative stress in plasma, erythrocytes and tissues.