5.1 ANTIOXIDANT ACTIVITY OF DIFFERENT LAYERS OF ONION AT TWO DIFFERENT STAGES OF MATURATION IN VITRO

5.1.1 Total Phenolic Content

Phenolic profiles have been reported mainly for fruits and vegetables, and their physiological activity has been thoroughly studied. Total phenolic content (TPC) was evaluated, as a function of 1) size of onion (big and small), 2) in inner layers and outer layers. Figure 5.1.1 shows the quantitative data of TPC of onion extracts expressed as mg gallic acid equivalent (GAE)/100g fresh sample. 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).

Our results are in good agreement with earlier reports, where TPC content in whole onion cultivars ranged between 42.95 to 129.62 mg/100g grown in India [Kaur et al., 2009] and Vadalia onions ranged between 73 to 180 mg/100g [Sellappan and Akoh, 2002]. In general, onions have been reported to contain a higher phenolic content than other vegetables [Velioglu et al., 1998], due to red colour with high content of anthocyanins [Desjardins, 2008]. It has also been reported that the red onion had higher TPC than the white onion (2.2 mg GAE/g dw versus 1.1 mg GAE/g dw) [Gokce et al., 2010].

5.1.2 Total Flavonoid Content

Figure 5.1.2 shows the quantitative data of total flavonoid content of onion extracts expressed as mg quercetin /100g fresh sample. Results showed 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). Difference was also observed between flavonoid content of outer layers, being
Figure 5.1.1

Figure 5.1.1. Total Phenolic Content (TPC) of outermost living layers and innermost layers of onion at two different stages of maturation. Concentration of TPC is expressed as mg GAE/100 fresh weight. Values represent mean ±SD. Significant difference * (p < 0.01) and ** (p < 0.001) in the phenolic content of outer layers with their respective inner layers of different sizes. # (p < 0.05) between both the outer layer extracts of onion.

Figure 5.1.2

Figure 5.1.2. Total Flavonoid Content of outermost living layers and innermost layers of onion at two different stages of maturation. Concentration of Total Flavonoid Content is expressed as mg quercetin/100gm fresh weight. Values represent mean ±SD. Significant difference * (p < 0.01) in the flavonoid content of outer layers with their respective inner layers of different sizes. ** (p < 0.05) between both the outer layer extracts of onion.
higher in smaller onion (p < 0.05). Our results are in good agreement with earlier reports, where difference in flavonoid content was observed in the different parts of the onion bulb [Beesk et al., 2010; Bilyk et al., 1984; Prakash et al., 2007].

In general, a higher flavonoid content of onion extracts is mainly due to the presence of various phenolic compounds (quercetin, kaempferol and luteolin), among them the two quercetin conjugates quercetin-3,4’-O-diglucoside (QDG) and quercetin-4’-O-monom glucoside (QMG) are the main flavonols, which make up to 80–85% of the total flavonoid content of onion extracts [Desjardins, 2008; Price and Rhodes, 1997; Rhodes and Price, 1996; Sellappan and Akoh, 2002]. Quercetin and kaempferol and their glycosides have been reported to be the most abundant flavonoids in the acid hydrolysed samples of onion [Chu et al., 2000; Price and Rhodes, 1997].

Difference in phenolic content could be explained on the basis of higher flavonoid content of the outer layers as compared to inner layers. Therefore, the outer living layers are a non-negligible, valuable by-product for the extraction of flavonoids. It has been investigated recently that in some onion cultivars the outer living layers contain up to more than ten-fold higher total flavonoid levels than the inner layers and up to more than three-fold higher total flavonoid levels than the dead dry outer scales, for instance in the yellow skinned cultivar ‘Ailsa Craig’ and ‘Borettana’ [Beesk et al., 2010].

However, this difference in the distribution of flavonoids being higher in the outer layers could be explained by the increased activity of light-induced enzyme phenylalanine ammonia lyase which catalyses the biosynthesis of flavonoids in the outermost localizing living cells of the whole onion bulb due to more exposure of sunlight than the outermost dry peel and innermost layers of the onion bulb portion [Friedman, 1997, Hirota et al., 1999]. The cells of outermost dead dried peel of onion are not influenced to sunlight, resulting in lesser biosynthesis of flavonoids, thus having flavonoid content lesser than the outermost living cells layer [Beesk et al., 2010].

It has also been reported that the ratio between the flavonols changes within the different parts of the whole onion bulb. In the inner layers QDG is the dominant...
flavonol glucoside, with higher contents than QMG. In the outermost living layers the ratio from QDG to QMG is nearly equal and low quercetin levels could be found in almost every onion. In the dry dead outer scales, quercetin is the major flavonoid of the analysed flavonoids and QDG shows the lowest levels of the three flavonoids. This distribution between the different parts could be a result of the degradation from QDG to QMG and quercetin from the inner to the outer layers by plant glucosidases and environmental factors [Beesk et al., 2010]. The red and yellow skinned cultivars are generally higher in flavonoids than the white skinned onions, but there are exceptions in some cultivars, too [Beesk et al., 2010].

5.1.3 Antioxidant Activity (AOA) by FRAP Assay

The mechanism of antioxidant action in vitro may involve direct inhibition of the generation of reactive oxygen species, or the scavenging of free radicals. We used FRAP (Ferric reducing antioxidant power) assay developed by Benzie and Strain [1996] because it is the only one that directly estimates the capacity of antioxidants or reductants in a sample and is based on the ability of the analyte to reduce Fe$^{3+}$/Fe$^{2+}$ couple. The reaction detects compounds with redox potentials of $<$0.7 V (the redox potential of Fe$^{3+}$-TPTZ), so the FRAP test is a reasonable screen for the ability to maintain redox status in cells or tissues.

The antioxidant activity (AOA) of onion extracts was estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe-(II) complex, Figure 5.1.3. 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 Fe$^{2+}$/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.
Figure 5.1.3

[Graph showing antioxidant activity (AOA) by FRAP assay of outermost and innermost layers of onion at two different stages of maturation.]

Figure 5.1.3. Antioxidant Activity (AOA) by using FRAP assay of outermost living layers and innermost layers of onion at two different stages of maturation. Concentration of AOA is expressed as µM Fe²⁺/g fresh weight. Values represent mean ±SD. Significant difference * (p < 0.01) in the flavonoid content of outer layers with their respective inner layers of different sizes. # (p < 0.05) between both the outer layer extracts of onion.

Figure 5.1.4

[Graph showing free radical scavenging activity by DPPH radical of outermost and innermost layers of onion at two different stages of maturation.]

Figure 5.1.4. Free Radical Scavenging Activity by using DPPH radical of outermost living layers and innermost layers of onion at two different stages of maturation. Compared with 10⁻⁵ and 10⁻⁶ M Quercetin. Concentration of FRSA is expressed as % DPPH Inhibition. Values represent mean ±SD. Significant difference * (p < 0.05) and # (p < 0.01) in the % DPPH Inhibition of outer layers with their respective inner layers of different sizes. Significant difference * (p < 0.05) between both the outer layer extracts of onion.
5.1.4 Free Radical Scavenging Activity (FRSA) using DPPH Radical

Phenolic compounds and flavonoids, which are widely distributed, have the ability to scavenge free radicals by single-electron transfer [Prakash et al., 2007], the extracts of red onion were also studied for their free radical scavenging activity (FRSA) assayed by DPPH• free radical (2, 2-diphenyl-1-picrylhydrazyl). The DPPH test is simple and rapid, used worldwide in the quantification of FRSA. The reaction is based on the colour decrease occurring when the odd electron of the nitrogen atom in DPPH• is reduced by receiving a hydrogen atom from antioxidant compounds. DPPH• is known as a stable free radical, but is sensitive to light, oxygen, pH and the type of solvent used [Ozcelik et al., 2003].

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), Figure 5.1.4. 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. There was no significant difference in FRSA of outermost layers of smaller onion when compared to 10⁻⁶ M quercetin, taken as standard. This indicates that quercetin, which is the main flavonoid is present significantly in higher amount in outer layers. Similar results were observed for FRSA, wherein % DPPH inhibition ranged from 5.07 to 11.36 of aged extracts whole bulb of some Italian Allium species [Nencini et al., 2011]. In our study, there was an observable correlation between high radical scavenging/antioxidant activity and high amounts of total phenolics and flavonoids of the onion extracts. This result indicates that the phenolic compounds of onion extracts contribute to their antioxidative properties.

5.1.5 Percent Scavenging Activity of Hydrogen Peroxide

Ability of the onion extracts to scavenge hydrogen peroxide is shown in Figure 5.1.5. 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%).

**Figure 5.1.5**

![Graph showing % scavenging of H$_2$O$_2$ for different extracts](image)

Figure 5.1.5. Percent Scavenging Activity of Hydrogen Peroxide of outermost living layers and innermost layers of onion at two different stages of maturation, along with quercetin (200 mg/L) taken as standard. Values represent mean ±SD. Significant difference * (p < 0.05) in the scavenging activity of outer layers of larger onion with its respective inner layers and outer layers of smaller onion. Significant difference # (p < 0.01) in outer layers of smaller onion with its respective inner layers.

### 5.1.6 Reducing Power Activity

Figure 5.1.6 shows the reducing power of the extracts. 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.

The scavenging of hydrogen peroxide and reducing capacity of whole onion extracts has been recently documented by various investigators [Benkeblia, 2005; Shon et al., 2004]. Variation in FRSA and H$_2$O$_2$ scavenging activity in different layers of onion might be due to variation in the quantities of quercetin in the various layers of onion extracts. Quercetin is known to exert multiple mechanisms including antioxidant activity, anti-inflammation, modification of signal transduction pathways, and
interactions with receptors and other proteins. *In vitro* and *in vivo* studies have proven that antioxidant properties of quercetin protect the diabetic erythrocytes from oxidation induced damage [Rizvi and Mishra, 2009] and also protect DNA of normal cells against oxidative damage [Papiez et al., 2008]. These compounds might contain one or more aromatic hydroxyl groups which exert its antioxidative effects in cells by chelating transition metal ions, catalyzing electron transport and scavenging free radicals [Hu et al., 1995]. Since phenolic compounds present in the extract are good electron donors, they may accelerate the conversion of H$_2$O$_2$ to H$_2$O [Ruch et al., 1989], thus showing high reducing power.

**Figure 5.1.6**

![Graph showing reducing power](image)

**Figure 5.1.6.** Reducing Power of outermost living layers and innermost layers of onion at two different stages of maturation, along with quercetin (10 µg/mL) taken as standard. Here, an increased absorbance is indicative of higher reducing power. Values represent mean ±SD. Significant difference *(p < 0.001)* in the reducing power of outer layers with their respective inner layers of different sizes. # *(p < 0.01)* between both the outer layers.

However besides phenolic compounds, organosulfur compounds such as S-propenylcysteine sulfoxide (major component), S-propylcysteine sulfoxide and S-methylcysteine sulfoxide and non-flavonoid compounds have been reported in onion to show alkylperoxyl radical scavenging activity and also responsible for most of its biological properties [Sawa et al., 1999; Corzo-Martinez et al., 2007]. Statistical analysis also showed that radical scavenging activity, reducing capacity and scavenging of H$_2$O$_2$ of outer layers significantly correlated with TPC of outer layers.
The triple synergistic action of phenols from onion in scavenging ROS, repairing DNA radicals and metal chelation has been reported [Prakash et al., 2007].

5.1.7 Hydroxyl radical scavenging activity

Hydroxyl radicals can be produced in cells by a variety of processes: such as phagocytosis, prostaglandin biosynthesis, ionizing irradiation and decomposition of lipid hydroperoxides. It is generally proposed that such a radical could be originated by Fenton-type reaction. The hydroxyl radical is an extremely reactive in biological systems and has been implicated as highly damaging species in free radical pathology, capable of damaging biomolecules of the living cells. Hydroxyl radicals generated by the Fenton reaction are known to cause oxidatively induced breaks in DNA strands to yield its open circular or relaxed forms and contribute to significant biological effects such as carcinogenesis, mutagenesis and cytotoxicity. They are reported to mediate the lethal cell injury in cultured hepatocytes [Halliwell et al., 1999].

Figure 5.1.7

![Graph showing hydroxyl radical scavenging activity](image)

Figure 5.1.7. Hydroxyl radical scavenging activity (HRSA) (%) of outermost living layers and innermost layers of onion at two different stages of maturation, compared with quercetin (10^{-4} to 10^{-6} M). Values represent mean ±SD. Significant difference * (p < 0.05) in the scavenging activity of outer layers of larger onion with its respective inner layers and outer layers of smaller onion. Significant difference # (p < 0.01) in outer layers of smaller onion with its respective inner layers.

All the onion extracts were studied for their HRSA and compared with quercetin of different concentrations (10^{-4} mol/L and 10^{-6} mol/L), Figure 5.1.7. 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. These results are correlated with FRSA, indicating that flavonoids are present significantly in higher amount in outer layers.

### 5.1.8 Metal Chelating Activity

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and is known as effective lipid oxidation pro-oxidant, which catalyses various oxidation reactions in biological systems [Shon et al., 2004]. It causes lipid peroxidation through the Fenton and Haber-weiss reaction [Halliwell and Gutteridge, 1990] and decomposes the lipid hydroxide into peroxyl and alkoxy radicals that can perpetuate the chain reactions [Halliwell, 1991]. Metal ion-chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage [Kumar et al., 2008]. Thus, metal chelation is an important antioxidant property because it reduces the concentration of the catalyzing transition metal in lipid oxidation. The ferrous ion chelating activity assay of any extract or compound actually measures the capacity of that extract or compound to bind the Fe\(^{2+}\) ion. Ferrozine can quantitatively bind with Fe\(^{2+}\) to form a stable complex, which is coloured red and has its absorbance maximum at 510 nm. The absorbance of this complex at 510 nm steadily decreases with the increase of the chelating activity of the chelating agent.

Figure 5.1.8 shows the obtained ferrous chelating abilities of onion extracts, which was 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. The results suggests that the ferrous ion chelating effects of outer layers of onion are higher than inner layers (p < 0.05) and can be considered as moderate metal chelator since its activities are approximately two times lesser than EDTA. Quercetin also showed metal chelating
activity with an inhibition of 43.6 % at 100 µg/mL, suggesting that flavonoids possess strong affinity toward iron ions. These compounds contain aromatic hydroxyl groups which exert its antioxidative effects in cells by chelating transition metal ions, catalyzing electron transport and scavenging free radicals [Hu et al., 1995].

**Figure 5.1.8**

![Graph showing metal chelating activity of outermost and innermost layers of onion at two stages of maturation, compared with quercetin and EDTA.](image)

Figure 5.1.8. Metal Chelating Activity (%) of outermost living layers and innermost layers of onion at two different stages of maturation, compared with quercetin (100 µg/mL) and EDTA (100 µg/mL). Values represent mean ±SD. Significant difference * (p < 0.01) and ** (p < 0.001) in the reducing power of outer layers with their respective inner layers of different sizes. ** (p < 0.01) between both the outer layers.

### 5.1.9 α-Amylase Inhibitory Activity

Recent nutritional investigations have reported that polyphenols may be able to modulate nutrient availability through the inhibition of digestive enzymes involved in lipid and starch breakdown, which could lead to beneficial effects on calorie intake, obesity [McDougall et al., 2009] and blood glucose control [Grussu et al., 2011]. α-Amylase is one of the main enzymes in human that is responsible for the breakdown of starch to more simple sugars thus the inhibitors of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption and obesity management by reducing sugar level in blood. The inhibition of α-amylase has been suggested as a strategy for diabetes control as they can decrease the attenuated postprandial plasma glucose levels by ultimately decreasing glucose release from
starch and improves the glucose tolerance in diabetic patients [Ali et al., 2006; Kwon et al., 2006]. It acts upon large polysaccharides (starch) at internal bonds. In recent years, natural sources of α-amylase inhibitor have received a lot of interest due to search for alternative to synthetic enzyme inhibitors such as acarbose, metformin and orlistat, which have been found to exhibit adverse effects, mild efficacy and can cause gastrointestinal distress as a side effect [Randhir et al., 2008]. Although there are citations of anti-hyperglycemic and anti-diabetic activity of some Allium species [Campos et al., 2003; Azuma et al., 2007; Nickavar and Yousefian, 2009], least attention was given on the activity of the different layers of onion extracts on in vitro α-amylase activity.

In the present study, inhibitory activity of different sizes and layers of onion against porcine pancreatic α-amylase were studied and compared with quercetin, Figure 5.1.9. 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). Since, phenolics play a role in mediating amylase inhibition and have the potential for type II diabetes management [McCue and Shetty, 2004, Chethan et al., 2008].

Phenolics are able to bind with the reactive sites of α-amylase and alter its catalytic effects, hence, the presence of phenolics and flavonoids in onion extracts would have contributed toward α-amylase inhibition. Our results corroborates with recent report of Tadera et al. [2006], showing several flavonoid compounds especially quercetin for their inhibitory activity against alpha amylase and that inhibitory activity increased appreciably with an increase in the number of hydroxyl group on the B ring. Epidemiological studies have also shown that the intake of certain types of flavonoids, including quercetin and myricetin are inversely associated with the risk of incident type II diabetes [Griffiths et al., 2002]. The structural requirements that flavonoids possess for controlling starch digestion to inhibit human α-amylase: (i) hydrogen bonds between the hydroxyl groups of the polyphenol ligands and the
catalytic residues of the binding site and (ii) formation of a conjugated \( \pi \)-system that stabilizes the interaction with the active site [Piparo et al., 2008].

**Figure 5.1.9**

![Graph showing \( \alpha \)-Amylase Inhibitory Activity (%)](image)

**Figure 5.1.9.** \( \alpha \)-Amylase Inhibitory Activity (%) of outermost living layers and innermost layers of onion at two different stages of maturation, compared with quercetin \( (10^{-4}\text{ to } 10^{-6} \text{ M}) \). Values represent mean ±SD. Significant difference * \( (p < 0.05) \) in the scavenging activity of outer layers of larger onion with its respective inner layers and outer layers of smaller onion. Significant difference # \( (p < 0.01) \) in outer layers of smaller onion with its respective inner layers.

**5.2 PROXIMATE COMPOSITION OF DIFFERENT LAYERS OF ONION EXTRACT AT TWO STAGES OF MATURATION**

Plants generally contain chemical compounds (such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides) known as secondary metabolites, which are biologically active [Soetan and Oyewole, 2009]. Plants are also known to have high amounts of essential nutrients, vitamins, minerals and fatty acids and fibre. Onions have a unique combination of three families of compounds that are believed to have salutary effects on human health – fructans, flavonoids and organosulfur compounds are the richest sources of flavonoids in the human diet [Manach et al., 1998]. Proximate analysis in plants gives valuable information and help to access the quality of the sample. It provide information on moisture content, ash content, volatile matter content, ash, fixed carbon etc. Previously proximate
composition of different varieties of onion and different organs of onion have been performed [Stajner and Popovic, 2009]. However, no literature information is available on the proximate composition of different layers of onion. It is important to identify and harvest at a specific maturity stage of the plant so that valuable components (at their highest levels) within the plant can be utilized.

5.2.1 Moisture Content

Moisture content % indicates the shelf life of the fresh plant and time of storage to avoid spoilage due to its susceptibility to microbial attack. Moisture content is among the most vital and mostly used measurement in the processing, preservation and storage of food [Onwuka, 2005]. Significant difference (p < 0.05) between both the outer layers was observed. Values showed significant difference (p < 0.01) in the % moisture content of outer layers when compared to inner layers of larger onion, Figure 5.2.1.

Figure 5.2.1

![Figure 5.2.1](image_url)

**Figure 5.2.1. Comparison of % moisture content of outermost living layers and innermost layers of onion at two different stages of maturation.** Values represent mean ±SD. Significant difference * (p < 0.05) between both the outer layers. Significant difference ** (p < 0.01) in the % moisture content of outer layers when compared to inner layers of larger onion.

5.2.2 Protein Content

Outer layer of onion extracts showed difference in the protein content with respect to their innermost bulbs in different sizes of onion. Significantly small sized onions had
higher protein content (in both inner bulb and outer layers) compared with larger onions. Higher moisture content and lower protein content of outer layers of onion might be due to the difference in the distribution of flavonoids with respect to different layers, Figure 5.2.2.

5.2.3 Total Ash Content

Ash in food contributes the residue remaining after all the moisture has been removed as well as the organic material (fat, protein, carbohydrates, vitamins, organic acid etc.) have been incinerated at a temperature of about 500°C. Ash content is generally taken to be a measure of the mineral content of the original food [Onwuka, 2005]. No significant difference in ash content of different onion extracts was observed, Figure 5.2.2.

5.2.4 Crude Fibre Content

Crude fibre in food or plant is an indication of the level of non-digestible carbohydrate and lignin. This low level is considered appropriate, because it aids absorption of glucose and fat. Although crude fibre enhances digestibility, its presence in high level can cause intestinal irritation, lower digestibility and decreased nutrient usage [Jimoh and Oladiji, 2005]. Crude fibre is made up largely of cellulose together with a little lignin which is indigestible in human [Onwuka, 2005]. No significant difference was observed in case of crude fibre of different layers and sizes of onion, Figure 5.2.2.

5.2.5 Fat Content

Lipid provides very good sources of energy and aids in transport of fat soluble vitamins, insulates and protects internal tissues and contributes to important cell processes. The crude fat content was almost similar in different samples of onion with no statistical difference, Figure 5.2.2.
5.2.6 Carbohydrate content

No significant difference were observed in case of carbohydrate content of outermost living layers and innermost layers of onion at two different stages of maturation, Figure 5.2.3.

Figure 5.2.2

Figure 5.2.2. Comparison of % protein, crude fibre, ash and fat content of outermost living layers and innermost layers of onion at two different stages of maturation. Values represent mean ±SD. Significant difference * (p < 0.05) between both the inner layers. Significant difference * (p < 0.05) and ** (p < 0.01) of outer layers when compared to inner layers of onion.

Figure 5.2.3

Figure 5.2.3. Comparison of % carbohydrate content of outermost living layers and innermost layers of onion at two different stages of maturation. Values represent mean ±SD.
5.3 EFFECT OF DIFFERENT LAYERS OF ONION EXTRACT ON OXIDATIVE STRESS BIOMARKERS IN ERYTHROCYTES SUBJECTED TO OXIDATIVE STRESS BY t-BHP IN VITRO

5.3.1 Percent Hemolysis

As anucleated cells with intrinsically poor repair mechanisms, erythrocyte makes a good model to test the antioxidant capacity of antioxidant compounds in the presence of an oxidation stimulus [Coimbra et al., 2006]. Erythrocytes are highly susceptible to oxidative damage due to the high cellular concentration of oxygen and haemoglobin: a potentially powerful promoter for the oxidative processes. Oxidative damage of erythrocytes membrane (lipid and protein peroxidation) compromise cell integrity, which may be implicated in hemolysis associated with some hemoglobinopathies, certain drugs, transition metal toxicity, radiation, and in conditions of deficiency in some erythrocyte antioxidant systems [Ko et al., 1997].

The antioxidant effect of the onion extract was tested on osmotic fragility of erythrocytes which have been oxidatively stressed by incubating with t-BHP (with and without onion extract) and quercetin, Figure 5.3.1. In the present study, incubation with t-BHP resulted in increase in oxidative hemolysis of erythrocytes as compared to control. Onion extracts showed significant protection of the erythrocyte from t-BHP induced oxidative hemolysis (inner layers 52.3% and outermost layers 43.5%) (p < 0.01). Quercetin also protected the erythrocytes from oxidative hemolysis, an effect which was concentration dependent (p < 0.01).

The ability of quercetin to incorporate into the hydrophobic core of the membrane bilayer improves the antioxidative effectiveness of flavonoid by causing a reduction in membrane fluidity and membrane stability, which further limit diffusion of free radicals [Arora et al., 2000]. Our results corroborate with the recent report in which quercetin was found to inhibit both neutrophil oxidative burst activity and protect erythrocytes against hemolysis by free radicals [Hapner et al., 2010]. Also, organosulfur compounds have been reported in onion to show alkylperoxyl radical
scavenging activity and also responsible for most of its biological properties [Sawa et al., 1999; Corzo-Martinez et al., 2007]. The variation in protective effect with respect to layers of onion extracts (p < 0.01) against oxidative hemolysis is found to be correlated with the antioxidant activity of different layers of onion extracts discussed above.

**Figure 5.3.1**

![Graph showing oxidative hemolysis](image)

**Figure 5.3.1.** Effect of onion extracts (outermost and inner layers) and quercetin on % oxidative hemolysis in t-BHP induced oxidative stressed erythrocytes. Values represent mean ± SD. *a* (p < 0.01) as compared to onion outermost layers and inner layers. *b* (p < 0.01) as compared to inner layers. Treatment with quercetin shows significant protection * (p < 0.01) at different concentrations against t-BHP induced oxidative hemolysis.

### 5.3.2 Erythrocyte Malondialdehyde content

Polyunsaturated fatty acids within the membrane, an oxygen rich environment, and iron-rich Hgb make reds cells susceptible to peroxidative damage [Claster et al., 1984]. ROS initiate lipid peroxidation reactions that lead to loss of membrane integrity and cell death [Baynes, 2005]. MDA, a highly reactive bifunctional molecule, is an end product of membrane lipid peroxidation has been shown to cross-link erythrocyte phospholipids and proteins. This process results in impairment of the membrane related functions that ultimately leads to diminished survival.
Subjecting erythrocytes to increased oxidative stress by incubating them with t-BHP caused a significant increase in MDA formation ($p < 0.01$). Increased erythrocyte MDA level is known to affect erythrocyte membrane lipid bilayer fluidity [Bryszewska et al., 1995]. A high concentration of MDA in erythrocyte is a marker of cellular oxidative damage observed in stress or pathological conditions including aging [Rizvi and Maurya, 2007]. Onion extract protected t-BHP induced lipid peroxidation, the effect was greater with outer onion layer compared to inner layer extract ($p < 0.01$). Presence of quercetin at different concentrations ($10^{-5}$ mol/L and $10^{-6}$ mol/L) in the incubation medium protected the erythrocytes from t-BHP induced oxidative stress as evidenced from the decrease in the level of MDA ($p < 0.01$), Figure 5.3.2.

Figure 5.3.2

Figure 5.3.2. Effect of onion extracts (outermost and inner layers) and quercetin (at concentrations $10^{-5}$ mol/L and $10^{-6}$ mol/L) on malondialdehyde (MDA) level in t-BHP induced oxidative stressed erythrocytes. Concentration of MDA is expressed as nmol/mL of packed erythrocytes. Values represent mean ± SD. $^a$ ($p < 0.01$) as compared with quercetin ($10^{-5}$ mol/L and $10^{-6}$ mol/L) and onion extract (outermost and inner layers). $^b$ ($p < 0.01$) as compared to onion outermost layers, inner layers, $10^{-5}$ mol/L and $10^{-6}$ mol/L. $^c$ ($p < 0.01$) as compared to inner layers.

5.3.3 Erythrocyte Glutathione content

Glutathione exists either in reduced GSH or oxidized GSSG forms and participates in redox reactions by the reversible oxidation of its active thiol. GSH may covalently
bind to proteins through a process called glutathionylation and can act as a coenzyme for various cell defense enzymes. It can thus directly scavenge free radicals or act as a substrate for GPx and GST during the detoxification of H$_2$O$_2$, lipid hydroperoxides and electrophilic compounds. In erythrocytes, the major antioxidant is GSH which protects important proteins such as spectrin the oxidation of which can lead to increased membrane stiffness [Carroll et al., 2006]. GSH not only supports antioxidant defense, but is also an important sulfhydryl buffer, maintaining –SH groups in Hgb and enzymes in the reduced state [Baynes, 2005].

**Figure 5.3.3**

![Figure 5.3.3](image)

**Figure 5.3.3.** Effect of onion extracts (outermost and inner layers) and quercetin (at concentrations $10^{-5}$ mol/L and $10^{-6}$ mol/L) on reduced glutathione (GSH) content in t-BHP induced oxidative stressed erythrocytes. Concentration of GSH is expressed as mg/mL of packed erythrocytes. *a* ($p < 0.01$) as compared to t-BHP, outermost layer and quercetin. *b* ($p < 0.01$) compared to outermost, inner layer, $10^{-5}$ mol/L and $10^{-6}$ mol/L quercetin. *c* ($p < 0.05$) compared to inner layers.

The induction of oxidative stress following incubation with t-BHP resulted in decrease in intracellular GSH content ($p < 0.01$). Dumaswala et al. [2001] observed that in vitro augmentation of the erythrocyte endogenous antioxidant reserve, especially GSH, provides protection against cell damage induced by oxidative stress. A higher demand for GSH causes increased degenerative oxidative modifications of proteins or lipids.
Onion extracts protected the erythrocytes against t-BHP induced GSH oxidation; however, this increase was significantly higher in the outermost living layers as compared to the inner layers (p < 0.05). Quercetin protected the erythrocytes against t-BHP induced oxidative stress at different concentrations (10^{-5} \text{ mol/L} \text{ and } 10^{-6} \text{ mol/L}) (p < 0.01), Figure 5.3.3. The strong biological antioxidant activity of the onion extracts against oxidative stress might be due the presence of polyphenolic flavonoids in onion [Shon et al., 2004; Prakash et al., 2007], which protects the cell not only by buffering free radicals but also by altering cell membrane properties [Arora et al., 2000; Pawlikowska-Pawlega et al., 2003].

5.3.4 Plasma Membrane Redox System

The plasma membrane regulates numerous aspects of cell physiology and signaling and also protects cells against oxidative stress. Analogous to the mitochondrial inner membrane, the plasma membrane redox system (PMRS) includes CoQ and multiple redox enzymes that are involved in electron transport and energy metabolism [Ly and Lawen, 2003]. Cells respond to oxidative stress by transferring electrons from NAD(P)H and ascorbate to extracellular free radicals and/or oxidants. Incubation with t-BHP resulted in increase in PMRS activity in erythrocytes as compared to control showing a state of oxidative stress (p < 0.01).

However, a significant decrease in the PMRS activity was observed in the erythrocyte from t-BHP induced oxidative stress when co-treated with onion extract (inner layers and outermost layers) (p< 0.01). Quercetin also resulted in reduced PMRS activity, thereby protecting the erythrocytes from oxidative damage, an effect which was concentration dependent (p < 0.01). These results corroborated with other reports showing that dietary antioxidants is important in maintaining levels of reduced CoQ and \( \alpha \)-tocopherol in the plasma membrane, thereby protecting the plasma membrane from lipid peroxidation [Villalba and Navas, 2003]. These results further suggests that the protective effect of onion extract on lipid peroxidation, GSH, erythrocyte hemolysis, and 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.
RESULTS AND DISCUSSION

Figure 5.3.4

![Graph showing PMRS activity with various treatments](image)

Figure 5.3.4. Effect of onion extracts (outermost and inner layers) and quercetin (at concentrations $10^{-5}$ mol/L and $10^{-6}$ mol/L) on plasma membrane redox system (PMRS) activity in t-BHP induced oxidative stressed erythrocytes. PMRS activity is expressed as $\mu$mol/mL of packed erythrocytes. Values represent mean ± SD. ***(p < 0.01) as compared to t-BHP. ***(p < 0.01) as compared to outermost, inner layer and quercetin. *(p < 0.01) as compared to inner layers.

5.4 EFFECT OF ONION EXTRACT ON OXIDATIVE STRESS BIOMARKERS IN ERYTHROCYTES SUBJECTED TO OXIDATIVE STRESS IN VIVO

5.4.1 Effect of Mercuric Chloride Treatment on Body Weight of Rats in a 30 Day Period

Oxidative stress is a deleterious condition that occurs when there is damage to cellular components, including proteins, lipids, and DNA [Graf et al., 2005]. Mercuric chloride ($\text{HgCl}_2$) is one of the most toxic forms of mercury and once absorbed into blood stream combines with proteins in the plasma or enters the red cells [Patrick, 2002]. Previous studies have shown that $\text{HgCl}_2$ toxicity in rats results in the production of ROS which in turn generates oxidative stress [Augusti et al., 2008].

In the present study, our observation of a marked reduction in body weight of rat exposed to $\text{HgCl}_2$ after 30 days is supported by previous published report [Rao and
Chhunchha, 2010]. Weight loss is known to be the basic aspect of mercury toxicity and has been attributed to reduced food intake. A significant (p < 0.05) reduction in body weight was observed after four weeks in HgCl₂ treated rats, Figure 5.4.1.

Figure 5.4.1

![Graph showing the effect of mercuric chloride (HgCl₂) treatment on body weight of rats in a 30 day period]

5.4.2 Plasma Antioxidant Capacity

5.4.2.1 Plasma Antioxidant Capacity by Frap Assay

The antioxidant capacity of plasma is the primary measure and a reliable marker to evaluate the extent of oxidative stress in induced pathological events. All the groups were studied for the plasma antioxidant capacity by FRAP assay. Treatment with either onion extract, catechin or quercetin increased the antioxidant potential of the plasma (p < 0.05) and (p < 0.01) with respect to control, however, the antioxidant potential of plasma was significantly (p < 0.001) reduced in rats treated with HgCl₂. Treatment of HgCl₂ challenged rats with either onion extract, quercetin or catechin significantly (p < 0.01) improved the antioxidant potential of the plasma compared to HgCl₂ treated group, Figure 5.4.2.1.
Figure 5.4.2.1

![Graph showing FRAP value comparison](image)

Figure 5.4.2.1. Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on total antioxidant capacity of plasma (measured in terms of FRAP value in vivo in wistar strain rat). FRAP value is expressed as μmol Fe (II)/L of plasma. Values represent mean ±SD. * (p < 0.05) and ** (p < 0.01) as compared to control. # (p < 0.01) as compared to HgCl₂. *** (p < 0.05) as compared to Onion+ HgCl₂, Catechin+ HgCl₂, Quercetin+ HgCl₂.

Figure 5.4.2.2

![Graph showing Radical Scavenging Activity comparison](image)

Figure 5.4.2.2. Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on DPPH Radical Scavenging Activity of plasma in vivo in wistar strain rat. Values represent mean ±SD. * (p < 0.05) and ** (p < 0.01) as compared to control. # (p < 0.01) as compared to HgCl₂. *(p < 0.05) as compared to Onion+ HgCl₂, Catechin+ HgCl₂, Quercetin+ HgCl₂.

The present study demonstrates a decrease in antioxidant capacity in terms of FRAP value in HgCl₂ treated rats, however supplementation with flavonoids caused improvement of antioxidant potential in HgCl₂ treated rats. The results are in
agreement with a previous report which shows that oral administration of flavonoids (quercetin) enhanced the antioxidative ability of rat plasma, indicating that conjugated metabolites participate in the antioxidant defence [Santos et al., 2008].

5.4.2.2 Plasma Radical Scavenging Activity (by using DPPH Radical)

Significantly elevated levels of radical scavenging activity of plasma (measured by using DPPH radical) were observed (p< 0.05 & p< 0.01), when treated alone with onion, quercetin and catechin groups. However, HgCl₂ treatment significantly depleted the radical scavenging activity (p< 0.001). Co-treatment of onion extract, quercetin and catechin with HgCl₂ significantly (p< 0.01) improved the radical scavenging activity as compared to HgCl₂ group, Figure 5.4.2.2.

5.4.3 Lipid Oxidation

5.4.3.1 Erythrocyte Lipid Peroxidation

Lipid peroxidation (LPO) is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane function, structural integrity, decrease in membrane fluidity and inactivation of a several membrane bound enzymes [Pandey and Rizvi, 2010a]. Under oxidative stress, an increased MDA content is an important indicator of lipid peroxidation. Increased level of MDA in erythrocyte has been reported in many disease conditions which are accompanied with oxidative stress [Pandey and Rizvi, 2010a].

At the end of fourth week, the level of MDA was significantly increased in the HgCl₂ group compared with the control (p< 0.001), and, in contrast, no statistically significant change was observed in rats treated with onion, catechin or quercetin. In this study, HgCl₂ treatment increased lipid peroxidation by generating free radicals. This toxicity might be due to mercury-induced alterations in membrane integrity via the formation of reactive oxygen species by successive hydroperoxide formation and β cleavage of polyunsaturated fatty acids in vivo or due to perturbation of antioxidant defense mechanisms. The MDA level was significantly decreased in erythrocytes at the end of the fourth week in the onion + HgCl₂, catechin + HgCl₂ group and quercetin + HgCl₂ group, as compared to the HgCl₂ group (p< 0.05) and (p <0.001), Figure 5.4.3.1.
Figure 5.4.3.1

Figure 5.4.3.1. Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on erythrocyte malondialdehyde (MDA) content in vivo in wistar strain rat. Concentration of MDA is expressed as nmol/mL of packed RBC. Values represent mean ±SD. # (p < 0.01) as compared to HgCl₂. * (p < 0.05) and ** (p < 0.01) as compared to HgCl₂.

Figure 5.4.3.2

Figure 5.4.3.2. Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on plasma malondialdehyde (MDA) content in vivo in wistar strain rat. Concentration of MDA is expressed as nmol/mL of plasma. Values represent mean ±SD. # (p < 0.01) as compared to control. *(p < 0.05), ** (p < 0.001) and † (p < 0.01) as compared to HgCl₂.

5.4.3.2 Plasma Lipid Peroxidation

Under oxidative stress, lipid peroxidation affects not only cellular lipids but also lipoproteins. Oxidative stress induced peroxidation of membrane lipids can be very
damage as it results in generation of a variety of relatively stable decomposition end
products which can then be measured in plasma as an indirect index of oxidative
stress.

In this study, HgCl₂ treatment increased lipid peroxidation by generating free radicals
resulting in significantly elevated levels of MDA in plasma (p < 0.01). However, the
plasma MDA level was significantly decreased at the end of the fourth week on
supplementation of onion extract, quercetin and catechin in oxidatively stressed rats
compared with the HgCl₂ group (p < 0.05; p < 0.001 & p < 0.01), Figure 5.4.3.2.

5.4.3.3 Plasma Low-Density Lipoprotein Oxidation

Previous studies have shown that HgCl₂ treatment of rats results in the production of
ROS, which in turn generates oxidative stress [Augusti et al., 2008]. Oxidative
modification of LDL takes place in the sub-endothelial space of the arterial wall and a
certain amount of OxLDL is released into the circulation with increased intracellular
formation of ROS resulting into OxLDL-induced formation of atherosclerotic plaques
[Nishi et al., 2002; Assinger et al., 2010]. The plasma OxLDL levels are
significantly elevated in patients with acute myocardial infarction, cerebral infarction
or chronic renal failure accompanied by hemodialysis. It was found that the plasma
OxLDL level increased prior to aortic atherosclerotic lesion enlargement in
apolipoprotein E-knockout mice [Itabe, 2012].

Supplementation of quercetin and catechin alone, significantly (p < 0.05) decreased
the plasma OxLDL levels as compared to control, onion extract showed little effect on
plasma OxLDL levels. 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, Figure 5.4.3.3.

The protection of lipoproteins (LDL and HDL) from oxidative modification might be
probably the result of the ability of paraoxonase to hydrolyze specific oxidized lipids
in oxidized lipoproteins and human atherosclerotic lesions [Aviram et al., 1998].
Figure 5.4.3.3

**A**

![Graph A](image)

Absorbance of Oxidised LDL at 245 nm

- Catechin
- Quercetin
- Onion
- Control

Time (in Second)

**B**

![Graph B](image)

Absorbance of Oxidised LDL at 245 nm

- Control
- HgCl₂
- Onion + HgCl₂
- Quercetin + HgCl₂
- Catechin + HgCl₂

Time (in Second)

**Figure 5.4.3.3.** Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on induced LDL oxidation measured as a function of increase in absorbance at 245 nm for 3000 seconds. Figure 5.4.3.3A shows effect without HgCl₂ compared with control and Figure 5.4.3.3B shows effect with HgCl₂ compared with control.

It was shown previously that covalent linkage of lipid peroxidation products to the LDL protein, as well as the accumulation of lipid peroxides in LDL, is diminished in the presence of PON1 [Sanvanich et al., 2003]. Dietary supplementation with
polyphenolic antioxidants inhibits LDL oxidation and macrophage foam cell formation and attenuates development of atherosclerosis in animals [Aviram et al., 1999]. The inhibition of LDL oxidation by polyphenols could be related, at least in part, to a direct effect of the polyphenols on the LDL, since both quercetin and catechin were found to bind to the LDL particle via the formation of an ether bond. We thus conclude that dietary consumption of onion or its polyphenolic flavonoids quercetin and, to a lesser extent, catechin is associated with reduced susceptibility of LDL to oxidation and aggregation. Thus, the combination of potent antioxidants (as indicated by increased radical scavenging activity of plasma and inhibition of lipid peroxidation with reduced plasma MDA levels) could possibly play a major role in reducing oxLDL.

Previous studies have shown that licorice-derived glabridin can preserve paraoxonase activity during lipoprotein oxidation [Aviram et al., 1999], and pomegranate juice consumption also increases PON1 arylesterase activity, in association with a reduction in LDL susceptibility to copper ion-induced oxidation [Aviram et al., 2000]. These results provide evidence of the positive effect of onion extract on prevention of LDL oxidation during periods of oxidative insult.

5.4.4 Protein Oxidation

The exposure of proteins to ROS can alter the physical and chemical structure of the target causing consequent oxidation of side-chain groups, protein scission, backbone fragmentation, cross-linking, unfolding and formation of new reactive groups [Dalle and Donne, 2006]. The latter include oxidation of hydrophobic amino acyl residues to hydroxy and hydroperoxy derivatives, protein carbonylation, oxidation of thiol (T-SH) groups, dityrosine formation and many others. The conformational changes that result from this complex of reactions lead to the decrease or loss of protein biological function. Prolonged exposure of protein to reactive molecules leads to spontaneous post synthetic modifications, such as glycation or oxidation; among other modifications, ROS can convert proteins to carbonyl derivatives by a variety of oxidative mechanisms [Stadtman, 1992], and protein carbonyls formed are considered sensitive indices of oxidative injury to proteins [Levine et al., 1994].
Protein carbonyls (PCO), advanced oxidation protein products (AOPP) and protein hydroperoxides (P-OOH) are the markers for protein oxidation mentioned in this study.

5.4.4.1 Protein Carbonyl

Maintenance of protein redox state is fundamental for cell function, whereas structural changes in proteins are considered to be among the molecular mechanism leading to progression and development of degenerative diseases [Altomare et al., 1997]. Protein carbonyl (PCO) is an important index of oxygen radical-mediated protein damage where oxidative modification of proteins may occur via various mechanisms: direct oxidation of amino acid side chains (especially of proline, arginine, lysine and threonine), modification of side chains with lipid peroxidation products (malondialdehyde, 4-hydroxy-2-nonenal), and with products of glycation and glycoxidation. Protein carbonyl derivatives thus, can also be generated through oxidative cleavage of proteins by either the α-amidation pathway or by oxidation of glutamyl side chains, leading to formation of a peptide in which the N-terminal amino acid is blocked by an α-ketoacyl derivative (Berlett and Stadtman, 1997). All of these mechanisms introduce a carbonyl group into a protein and may lead to the loss of its biological activity [Berlett and Stadtman, 1997]. The damaging effect of protein oxidation is in most cases quite irreversible. Therefore, PCO may serve as a biomarker of general oxidative stress.

In this study, supplementation of flavonoids (catechin and quercetin) alone resulted in reduced protein oxidation (p< 0.05). HgCl₂ treatment resulted in significantly elevated levels of PCO in erythrocytes membrane of rats as compared to control (p< 0.001). However, PCO level was significantly decreased at the end of the fourth week on supplementation of onion extract, quercetin and catechin in erythrocytes membrane of oxidatively stressed rats compared with the HgCl₂ group (p <0.001), Figure 5.4.4.1.

In this study, HgCl₂ treatment increased protein carbonyl formation by generating free radicals. This toxicity may be due to mercury-induced alterations in membrane integrity via the formation of ROS by successive hydroperoxide formation and β cleavage of polyunsaturated fatty acids in vivo or due to perturbation of antioxidant
defense mechanisms. Hg(II) can also inactivate a number of enzymes by blocking the functional sites through binding to sulfhydryl groups, which are part of catalytic or binding domains. Covalent binding to sulfhydryl groups by Hg(II) alters protein conformation, creates protein adducts through modification of side chains and finally leads to changes in protein shape and activity [Sanders et al., 1996]. Accumulation of modified proteins disrupts cellular function, either by loss of catalytic and structural integrity or by interruption of regulatory pathways [Dean et al., 1997; Kayali et al., 2007; Stadtman and Levin, 2000].

Figure 5.4.4.1

![Figure 5.4.4.1](image)

**Figure 5.4.4.1.** Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on protein carbonyl (PCO) content in vivo in erythrocyte membrane of wistar strain rat. Concentration of PCO is expressed as nmol/mg protein. Values represent mean ±SD. * (p < 0.01) as compared to control. **(p < 0.001) as compared to HgCl₂. # (p < 0.001) as compared to Onion+HgCl₂, Catechin+HgCl₂, Quercetin+HgCl₂.

Increased levels of PCO in oxidatively stressed rats were scavenged by the onion extracts rich in flavonoids and polyphenols. Several studies have shown that these compounds (flavonoids and polyphenols) inhibited the formation of free radicals and propagation of free radical reactions through hydrogen-donation and aromatic hydroxylation [Jovanovic et al., 1994] and/or through the chelation of transition-metal ions [Paganga et al., 1996; Brown et al., 1998]. The free radical scavenging properties of these compounds could protect the human body from free radical-mediated diseases and reduce the risk of cancer, inflammation and diabetes [Verma et al., 1998]. Thus, supplementation of onion extract, alongwith quercetin and catechin protected the erythrocyte membrane from HgCl₂ induced oxidative stress, as evidenced by the decrease in PCO level at the end of four weeks.
5.4.4.2 Advanced Oxidation Protein Products

Witko-Sarsat et al. [1998] described a novel family of oxidized protein compounds, which were designated advanced oxidation protein products (AOPP). AOPP, considered as a novel marker, correspond to highly oxidized proteins and specifically to albumin and formed during oxidative stress by reaction of plasma proteins with chlorinated oxidants. They are defined as dityrosine containing cross-linked protein products and are considered as reliable markers to estimate the degree of oxidative modifications of proteins [Witko-Sarsat et al., 1998]. AOPP are predominantly aggregates of albumin damaged by oxidative stress [Kalousova et al., 2005]. In vivo plasma levels of AOPP closely correlate with levels of neopterin, a marker of macrophage activation state, as well as with dityrosine, a hallmark of oxidized proteins and pentosidine, an advanced glycation end products [Witko-Sarsat et al., 1998]. Skvarilova et al. [2005] showed that patients with acute coronary syndromes have increased levels of AOPP and that the plasma concentration of these products differs significantly from healthy volunteers.

The importance of AOPP has been highlighted: these products in fact may act as proinflammatory mediators and may trigger activation of oxidative “bursts” in neutrophils, monocytes and T-lymphocytes [Alderman et al., 2002]; raised AOPP levels, independently from fibrinogen and from C reactive protein (CRP) levels, predicted atherosclerotic cardiovascular events in patients with elevated atherothrombotic risk [Descamps-Latscha et al., 2005].

At the end of fourth week, supplementation of flavonoids (quercetin) alone resulted in reduced AOPP levels as compared to control (p< 0.05 & p<0.01). In contrast, occurrence of protein oxidative stress by HgCl₂ treatment in experimental rats was confirmed by increased AOPP levels in plasma of rats as compared to control (p< 0.001) , which reflected an excess of free radical generation and of protein oxidative damages [Gallan et al., 2003], Figure 5.4.4.2.

The AOPP level was significantly decreased in the onion + HgCl₂, catechin + HgCl₂ group and quercetin + HgCl₂ group compared with the HgCl₂ group (p< 0.001). The increased levels of AOPP in oxidatively stressed rats were scavenged by the onion.
extracts rich in flavonoids and polyphenols, indicating that onion extract, by decreasing oxidative stress, may be effective in preventing oxidative protein damages which are thought to be involved in progression of various degenerative diseases.

**Figure 5.4.4.2**

![Figure 5.4.4.2](image)

**Figure 5.4.4.2.** Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on advanced oxidation protein products (AOPP) content *in vivo* in plasma of wistar strain rat. Concentration of AOPP is expressed as µmol/L plasma. Values represent mean ±SD. * (p < 0.01) as compared to control. ** (p < 0.05) as compared to onion and catechin. # (p < 0.001) as compared to control, Onion+ HgCl₂, Catechin+ HgCl₂, Quercetin+ HgCl₂.

**5.4.4.3 Protein Hydroperoxides**

A significant consequence of free radical damage to proteins is the formation of protein hydroperoxides (P-OOHs) [Gebicki, 1997]. Proton abstraction by hydroxyl or peroxyl radicals on amino acid residue side chains produces a carbon centred radical, which rapidly reacts with oxygen and hydrogen ions to form a protein hydroperoxide. Subsequent work has shown that many proteins can be peroxidized by physiologically important ROS, that the resultant P-OOHs can oxidize some biological anti-oxidants and that they can be a source of secondary free radicals [Davies et al., 1995; Lacsamana and Gebicki, 1996]. These findings suggest that P-OOHs may constitute important links in the chain of damage initiated by the ROS under physiological conditions [Gebicki, 1997]. Their potential to cause biochemical damage was confirmed by their formation in tissues which was provided by the finding of unique hydroxylated derivatives of the six amino acids known to be highly susceptible to peroxidation [Gebicki and Gebicki, 1999]. These are derived from the parent amino acid hydroperoxides generated by the action of ROS on proteins. Thus, the recent
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finding of hydroxyleucine and hydroxyvaline in human atherosclerotic plaques [Fu et al., 1998], provides strong evidence for the formation of P-OOHs under physiological conditions in tissues subjected to oxidative stress.

The formation of protein hydroperoxides occurs in a different way than protein carbonyls and AOPPs. It is well established that the P-OOHs formed particularly on aliphatic amino acid residues (Val, Leu, Ile, Glu, Pro, Lys) are generated under the absolute requirement of the oxygen presence [Davies et al., 1995; Gebicki and Gebicki, 1999]. The P-OOHs may be reductively detoxified to hydroxides but their decomposition can also result in the formation of further radicals that may propagate reaction chains [Davies et al., 1995; Gebicki and Gebicki, 1999]. PON1’s free thiol group is supposed to be the active site for its antioxidant activity [Jaouad et al., 2006]. The thiol (-SH) moiety on the side chain of the amino acid cysteine is particularly sensitive to redox reactions and is an established redox sensor [Eaton, 2006]. Protein thiol groups are also readily oxidised by POOHs with this potentially resulting in enzyme inactivation [Morgan et al., 2005].

Figure 5.4.4.3

[Graph of Plasma Protein Hydroperoxides (nmol/mg plasma) for different treatments including Control, Onion, Catechin, Quercetin, HgCl₂, Onion + HgCl₂, Catechin + HgCl₂, and Quercetin + HgCl₂ with statistical significance levels indicated by asterisks and hash symbols.]

Figure 5.4.4.3. Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on protein hydroperoxides (P-OOH) content in vivo in plasma of wistar strain rat. Concentration of P-OOH is expressed as nmol/mg plasma. Values represent mean ±SD. *** (p < 0.05) as compared to onion, catechin and quercetin. ** (p < 0.001) as compared to control. # (p < 0.05) as compared to HgCl₂. * (p < 0.01)) as compared to HgCl₂.

Though relatively stable, P-OOHs react with DNA to form protein-DNA cross-links, oxidise cellular thiol groups, and consume the key cellular antioxidants ascorbate and glutathione [Gebicki, 1997]. As a reactive species, P-OOHs are considered to be a...
major source of oxidative stress after exposure to free radical flux [Gebicki, 1997; Fu et al., 1998]. Mildly oxidised proteins are readily degraded and removed from the cell but when proteins are highly oxidised they aggregate and/or their solubility decreases which makes their elimination difficult [Cecarini et al., 2007; Grune et al., 2003]. These highly oxidised proteins may contribute to several neurodegenerative diseases such as Alzheimer and may be involved in the ageing process [Grune et al., 2003; Widmer et al., 2006]. Therefore, P-OOHs can be a useful markers of oxidative stress. It has been investigated recently that elevated protein hydroperoxide levels may also provide an effective and stable marker of oxidative stress during aging that may predispose towards many disease and stress conditions [Mehdi and Rizvi, 2013].

In this study, administration of onion extract and flavonoids (catechin and quercetin) alone resulted in reduced P-OOH formation ($p<0.05$). HgCl$_2$ treatment resulted in significantly elevated levels of P-OOH in plasma of rats as compared to control ($p<0.001$). However, P-OOH level was significantly decreased at the end of the fourth week on supplementation of onion extract, quercetin and catechin in plasma of oxidatively stressed rats compared with the HgCl$_2$ group ($p<0.01$ & $p<0.05$), Figure 5.4.4.3.

5.4.4.4 Plasma total thiol

Plasma Thiol (−SH) Levels include free amino thiols such as GSH, cysteine and homocysteine and protein bound thiols, which are a natural reservoir of the reductive capacity of the cell. They are extraordinarily efficient antioxidants that can preserve the correct structure of proteins, and can protect cells, and thus the body, from damage induced by free radicals [Kondo et al., 2009; Pandey et al., 2010c]. The oxidation of plasma −SH group, termed as thiol stress is quantitatively the major manifestation of protein oxidation.

Plasma −SH Levels are used to identify clinical conditions with excess of free radical generation, since in these settings the conformation of albumin is altered, allowing T-SH groups to be oxidized [Cakatay et al., 2008]. The most significant out of the multifarious roles played by Thiols (−SH) in vivo is their function as components of
the intracellular and extracellular redox buffer. –SH groups play a prominent role in antioxidant reactions and also in reactions of catalysis, regulation, electron transport and those preserving the correct structure of proteins [Rokutan et al., 1994]. Thus, reduced cellular levels of –SH can be a diagnostic indicator of a pathological status. The levels and mutual relations between different redox forms of –SH in plasma (realized through redox reaction including thiol disulfide exchange) are decisive for the plasma redox capacity. Glutathione, cysteine and homocysteine are present in plasma mostly in the form of symmetrical and mixed disulfides and also as a protein bound fraction. Following severe oxidative stress, both the decrease in the proteolytic degradation and the accumulation of non-folded proteins may be the cause and/or the consequence of many disorders [Rizvi and Maurya, 2007; Gornik and Lauc, 2008]. Thus, plasma –SH may be considered not only as metabolites and transported between organs and tissues, but also as functionally important plasma components [Iciek et al., 2004].

Figure 5.4.4.4

In this study, administration of onion extract and flavonoids (catechin and quercetin) alone resulted in plasma -SH formation (p< 0.05). A significant (p< 0.01) depletion of plasma -SH in HgCl2 treated rats demonstrates that a condition of oxidative stress prevails in rats as a result of mercury toxicity, Figure 5.4.4.4. The reason being that mercury has strong affinity for thiol (-SH) binding groups, especially on endogenous
bimolecules thus depleting intracellular thiols, and causing cellular oxidative stress or predisposing cells to it and forming free radicals which may further increase lipid peroxidation [Hultberg et al., 2001]. We observe a protective effect of onion extract, catechin and quercetin against HgCl₂ induced oxidative stress evidently through marked reduction in lipid peroxidation and increased plasma -SH levels. It is possible that the antioxidant components of the extracts might act as sacrificial antioxidants sparing the depletion of endogenous -SH during HgCl₂ induced oxidative stress. The negative correlation between plasma total thiols and AOPP observed in HgCl₂ treated rats indicates that the protein oxidation and hence AOPP increases as the protective antioxidant activity of total thiols decrease.

### 5.4.5 Intracellular Reduced Glutathione

Reduced glutathione (GSH) is a primary intracellular antioxidant present in almost all living cells including erythrocytes, it is considered as a biomarker of redox imbalance at cellular level. The glutathione antioxidant system plays a fundamental role in cellular defence against reactive free radicals and other oxidant species. GSH depletion has been shown to intensify lipid peroxidation and predispose cells to oxidant damage [Pandey and Rizvi, 2010a].

Treatment with onion extract and catechin caused slight increase (13.92 % and 20.70 %) in GSH level (p <0.05), however, treatment with quercetin resulted in significantly higher GSH level (37.02 %) as compared to control (p< 0.001). HgCl₂ treatment significantly depleted erythrocyte GSH as compared to control (p< 0.001). Cotreatment of onion extract, catechin and quercetin with HgCl₂ significantly (p< 0.001) improved the GSH level as compared to HgCl₂ group, 5.4.5.

A significant depletion of erythrocyte GSH in HgCl₂ treated rats demonstrates that a condition of oxidative stress prevails in rats as a result of mercury toxicity. A single Hg ion can bind to and cause irreversible excretion of up to two GSH molecules. The reason being that mercury has strong affinity for thiol -SH binding groups, especially on endogenous biomolecules [Hultberg et al., 2001]. Released Hg ions form complexes with GSH thereby disturbing its metabolism [Patrick, 2002]. As a result of binding of mercury to glutathione and subsequent elimination of intracellular
glutathione, levels of GSH are lowered in the cell and which manifests as decrease in the antioxidant potential of the cell.

**Figure 5.4.5**

The results on the oxidative imbalance after mercury intoxication corroborates with many studies showing elevated values of MDA with decreased GSH contents in kidney and other tissues [Augusti et al., 2008; Rao and Chhunchha, 2010]. In a significant report blood is shown to reflect tissue oxidative stress with respect to MDA and GSH [Veskoukis et al., 2009]. We observe a protective effect of onion extract against HgCl₂ induced oxidative stress evidently through marked reduction in lipid peroxidation and increased GSH content of erythrocytes and antioxidative activity of plasma. This is in agreement with previous studies which show that supplementation of onion rich diet results in increase in antioxidative ability of rat plasma [Azuma et al., 2007]. The present observation may be explained due to the presence of flavonoids in red onion (especially quercetin). It is possible that the antioxidant components of the extracts might act as sacrificial antioxidants sparing the depletion of endogenous GSH during HgCl₂-induced oxidative stress. Furthermore, dietary polyphenols have been shown to upregulate the expression of c-glutamylcysteine synthetase, the rate-limiting enzyme in the biosynthesis of GSH.
This may explain, in part, the enhancement of GSH in HgCl$_2$ exposed rats treated with onion extracts. The high antioxidant properties of onion extract could also be attributed to the rich presence of organosulfur containing active compounds in the form of cysteine derivatives (S-methylcysteine sulfoxide) which is a rate limiting substrate in GSH biosynthesis and has also been found to be effective in preventing or ameliorating oxidative stress by scavenging free radicals [El-Demerdash et al., 2005].

The present study demonstrates that oral administration of quercetin and catechin can effectively inhibit lipid peroxidation in vivo, and increase antioxidant capacity in control as well as HgCl$_2$-induced oxidative stressed rats. Such protection may be due to stabilization of the erythrocyte membrane owing to the incorporation of antioxidant thereby preventing physical damage of the membrane and resulting in more efficient free radical scavenging [Lopez-Revuelta et al., 2006]. It also involves indirect activation of transcription factors (e.g., Nrf2) that regulate the expression of genes encoding for antioxidant enzymes [Arredondo et al., 2010].

Catechins are reported to be scavengers of superoxide radicals, peroxyl radicals and inhibitors of lipid peroxidation [Demir et al., 2011]. It chelates iron and offers superoxide scavenging and lipid peroxidation lowering properties through its structural features (catechol group in ring B and a hydroxy group in ring C). In addition, dietary flavonoids interact with phase I and phase II enzyme system, thereby modulating expression of an important enzyme glutamylcysteine synthetase, which is responsible for the synthesis of glutathione [Moskaug et al., 2005]. Recently, protective effect of catechin and quercetin has been highlighted in chlorpyrifos induced toxicity in rat testis tissues [Kalender et al., 2012].

5.4.6 Plasma Ascorbic acid

Ascorbic acid is a major hydrophilic antioxidant in both plasma and the cytosol of many cells. It contributes to the neutralization of many water-soluble oxidants and acts synergistically with vitamin E to terminate radical induced lipid peroxidation. Ascorbic acid plays an important role in the synthesis and posttranslational
modifications of collagen. It is also necessary for the regulation of many cellular biochemical processes, including the scavenging of free radicals [Yue et al., 1989]. In this study, administration of flavonoids (catechin and quercetin) alone resulted in increased plasma ascorbic acid level (p< 0.05). A significant (p< 0.001) depletion of plasma ascorbic acid in HgCl₂ treated rats demonstrates that a condition of oxidative stress prevails in rats as a result of mercury toxicity. Supplementation of HgCl₂ challenged rats with onion extract, quercetin or catechin significantly (p< 0.05 & p< 0.01) improved the ascorbic acid level of the plasma compared to HgCl₂ treated group, Figure 5.4.6.

**Figure 5.4.6**

![Graph showing the effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on plasma ascorbic acid level in wistar strain rat.](image)

5.4.7 Plasma Sialic Acid

Sialic acid (SA), a family of acetylated or glycosylated derivatives of neuraminic acid--is a component of many glycoproteins and glycolipids, typically present at the outermost end of glycan chains of all cells [Schauer, 2009]. In the erythrocyte membrane, it is mainly contained in the SA-rich glycoporphins, mediating or modulating a variety of normal and pathological processes [Varki, 2008]. Due to negative charge and hydrophobicity, sialic acids have many structural and modulatory roles. They affect the action of some hormones, the recognition of different compounds, the cellular adhesiveness, the catalytic properties of enzymes, the transport process and antigenicity [Schauer, 2009].
Figure 5.4.7

Figure 5.4.7. Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on plasma sialic acid level in wistar strain rat. Concentration of plasma sialic acid is expressed as µM. Values represent mean ±SD. **(p < 0.001) as compared to HgCl₂, * (p < 0.05) as compared to Onion+ HgCl₂ and Catechin+ HgCl₂. ♯ (p < 0.01) as compared HgCl₂.

Plasma sialic acid level was significantly increased in HgCl₂ treated rats as compared with normal control rats (p< 0.001), Figure 5.4.7. HgCl₂ induced oxidative stress, resulted in modifications of erythrocyte membrane thereby causing increased levels of plasma sialic acid due to detachment or degradation of sialic acid from the erythrocyte membrane, causing changes in cell function or activity. The plasma sialic acid level has also been considered as an important parameter in identifying subjects prone to develop type-2 diabetes [Gavella et al., 2003]. Treatment with onion extracts, catechin and quercetin, restored cellular sialic acid with decreased plasma sialic content to the same levels as in control (p< 0.01 & p< 0.05). The role of onion extract in protecting against diabetes has been reported previously [Azuma et al., 2007] and a decrease in plasma sialic acid shown in this study is the novel finding as an indicative of a decrease progression of various degenerative diseases including diabetes and aging.

5.4.8 Antioxidant Enzyme Paraoxonase 1

Treatment with onion extract caused an increase (7.7 %) in PON1 activity (p< 0.05), treatment with quercetin resulted in significantly higher PON1 activity as compared to control (p< 0.001). We observe a significant decrease in PON1 activity (p< 0.0001) in HgCl₂ induced oxidatively stressed rats. However, 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. Catechin showed no significant increase in PON 1 activity when given alone and also upon supplementation to oxidatively stressed rats, Figure 5.4.8.

**Figure 5.4.8**

![Graph showing PON1 Arylesterase activity](image)

Figure 5.4.8. Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on paraoxonase 1 (PON 1) arylesterase activity in wistar strain rat. Concentration of PON1 is expressed as U/mL plasma. Values represent mean ±SD. **(p < 0.001) as compared to quercetin and HgCl₂. * (p < 0.05) as compared to control. # (p < 0.05) and *** (p < 0.01) as compared HgCl₂.

In the present study, PON1 activity was significantly reduced in HgCl₂ treated group when compared to the control group. The decreased serum PON1 activity could be related to the oxidative stress generated by HgCl₂. It is well known that PON1 loses its activity in an oxidative environment. Therefore, any factor that affects the status of oxidative stress will also affect PON1 activity [Gong et al., 2009]. Reduced PON1 activity is observed in several chronic diseases, including type 1 and 2 diabetes, hypercholesterolemia and during human aging [Mehdi and Rizvi, 2012; Ikeda et al., 2009]. Reduced serum PON1 activity and increased oxidative stress was also shown in E₀ mice and in dyslipidemic obese mice [Mertens et al., 2003]. Thus, PON1 activity has been suggested to play antioxidant defense role [Thomas-Moya et al. 2006]. Our study suggests that the increase in the susceptibility of LDL for oxidation is due to the decrease in serum PON1 arylesterase activity subsequent to oxidative stress generated by HgCl₂.
The present study demonstrates, for the first time that the oxidatively stressed rats supplemented with onion extracts showed increased PON1 activity, though not to the levels of the control group. These results demonstrate that the constituents of onion extracts (mainly quercetin) may be able to modulate the expression level of PON1 and enhance its activity, which is inactivated by oxidative stress. Our present study also demonstrates that quercetin significantly up-regulates PON1 activity to the greater level in oxidatively stressed rats. Our findings are supported by the report showing that dietary supplementation with quercetin up-regulates PON1 expression in the liver of laboratory mice [Boesch-saadatmandi et al., 2010]. In vivo studies have revealed an increase of PON1 serum activity following treatment with flavonoids [Aviram et al. 2000; Fuhrman and Aviram, 2002; Gouédard et al., 2004]. Unlike quercetin, catechin showed little effect on PON1 activity, despite the fact that catechins are known to be relatively potent free radical scavengers in vitro [Iacopini et al., 2008], indicating that the free radical scavenging properties of flavonoids in vitro do not seem to be positively associated with their PON1-inducing activity. Among various dietary polyphenolic compounds, catechin was a poor inducer of PON1 mRNA and PON1 transactivation as indicated in studies [Gouédard et al., 2004; Schrader et al., 2012].

5.4.9 Plasma Membrane Redox System

The PMRS is increasingly recognized as a major mechanism for reducing plasma membrane associated oxidative stress and, in compensating for mitochondrial dysfunction, as an alternative source of ATP production by increasing NAD levels and glycolysis. It has been reported that PMRS is protective mechanism that operates to maintain the ascorbate level in plasma which is crucial for maintaining the redox balance [Rizvi et al., 2006]. A marked increase in erythrocyte PMRS activity was observed in HgCl₂ challenged rats as compared to control (p< 0.001), in contrast, no significant activation of erythrocyte PMRS activity were observed when treated alone with onion, catechin and quercetin. A significant decrease in the PMRS activity was observed in HgCl₂ challenged rats when co-treated with onion extract (p< 0.01), catechin or quercetin (p< 0.001) compared to HgCl₂ group, Figure 5.4.9.
The higher activity of red cell PMRS in HgCl$_2$ treated rats is the result of generation of oxidative stress. Our observation of the reduction of PMRS activity upon supplementation by onion extract, catechin and quercetin suggests that such treatment enhances plasma antioxidant capacity and mitigates oxidative stress in HgCl$_2$ treated rats. On the basis of PMRS results, it is apparent that quercetin is more powerful antioxidant compared to catechin and onion extract.

**Figure 5.4.9**

![Figure 5.4.9](image)

**Figure 5.4.9.** Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl$_2$) induced oxidative stress on erythrocyte plasma membrane redox system (PMRS) in wistar strain rat. PMRS activity is expressed as μmol ferrocyanide/mL packed RBC/30 min. Values represent mean ±SD. # (p < 0.001) as compared to HgCl$_2$. ***(p < 0.01) as compared to HgCl$_2$. * (p < 0.001) as compared to Catechin+ HgCl$_2$ and Quercetin+ HgCl$_2$.

### 5.4.10 Acetylcholine Esterase activity

In the present study, the erythrocyte AChE activity was markedly decreased after HgCl$_2$ treatment showing an increase of oxidative stress in rat erythrocytes (p< 0.001), Figure 5.4.10. AChE in blood cells is biochemically identical to the enzyme contained in neurons and reveals lower individual dispersion as well as higher resistance towards external factors. Erythrocyte AChE plays an important role in the preservation of the integrity of the red cell. AChE is found to be an excellent enzymatic marker for RBC aging in man [Prall et al., 1998]. Furthermore, several studies have shown an increase in AChE activity in erythrocytes of Type 2 DM patients [De Bona et al., 2011].
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Figure 5.4.10

Figure 5.4.10. Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl$_2$) induced oxidative stress on acetylcholine esterase (AChE) activity in erythrocyte of wistar strain rat. AChE activity is expressed as μmol ACTC/ min/ mg protein. Values represent mean ±SD. **(p < 0.001) as compared to HgCl$_2$. * (p < 0.01) as compared to Onion+ HgCl$_2$, Catechin+ HgCl$_2$ and Quercetin+ HgCl$_2$.

However, supplementation of onion extract, quercetin and catechin improved AChE activity in erythrocyte membrane of oxidatively stressed rats (p< 0.01). These results may be attributed to the ability of onion extract in protecting the erythrocyte from free radical- mediated toxic damages and reduction of the oxidative stress. Our results are corroborated with a recent report reflecting beneficial effects of garlic oil intake through the significant activation of AChE enzymes [Hassan et al., 2010].

5.5 EFFECT OF ONION EXTRACT ON OXIDATIVE STRESS BIOMARKERS IN BRAIN AND LIVER TISSUES SUBJECTED TO OXIDATIVE STRESS IN VIVO

Oxidative stress is caused by ROS such as O$_2$ $^\cdot$-, H$_2$O$_2$, and OH $^\cdot$, and it causes changes in physiological systems in the body [Umakoshi et al., 2011]. ROS are converted to nontoxic substances by superoxide dismutase (SOD), glutathione peroxidase (GPx,) and catalase (CAT). SOD converts O$_2$ $^\cdot$- to H$_2$O$_2$, which then detoxified to H$_2$O by GPx and catalase [Bang et al., 2009]. It is well known that the liver metabolizes a wide range of both exogenous and endogenous compounds, and acts as a good indicator of detoxification processes taking place in the body. Liver plasma membrane
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plays a major role in hepatobiliary transport of biliary components and xenobiotics. There is a need for healthy signaling and message flow to maintain a good communication and coordination of biochemical process within the cell. Alterations in the plasma membrane of liver in its composition and fluidity can influence cancer mediated transport process and membrane bound enzyme activities. Injury to cell membrane by free radicals has been a recent focus since the vital activities of the cell are challenged [Poli, 2000].

The central nervous system is highly susceptible to oxidative stress. The vulnerability of the brain to oxidative stress induced by oxygen free radicals seems to be because the brain utilises about one-fifth of the total oxygen demand of the body for oxidative phosphorylation to acquire energy and that as it has a relatively small antioxidant capacity [Saeed et al., 2007], the brain cannot neutralize the toxic effects of free radicals. Furthermore, the brain contains a high concentration of easily peroxidisable fatty acids, and it is known that certain regions of the brain are highly enriched in iron, a metal that is catalytically involved in the production of damaging oxygen free radical species when it is in free form [Sudhakara et al., 2012, Halliwell and Gutteridge, 1999].

Previous studies revealed that HgCl₂ caused histopathological and ultrastructural lesions evidenced by preiportal fatty degeneration and cell necrosis in the liver [Stacchiotti et al., 2004]. In addition, Orr and blakley [1997] considered that such necrotic lesions may be due either to a progressive degenerative action of intracellular enzymes of the injured cells or to the metabolic disturbance and inhibition of protein and carbohydrate synthesis in the hepatic cells. HgCl₂ has a multitude of molecular, subcellular and cellular targets in the central nervous system [Gasso et al., 2001].

5.5.1 Effect on Enzymatic and Non enzymatic Antioxidants

5.5.1.1 Catalase

CAT are the most important defence mechanisms against toxic effects of ROS. CAT helps in the removal of H₂O₂ formed during the reaction catalyzed by SOD [Liu et al., 2010]. Treatment with onion extract, quercetin and catechin caused slight increase in CAT activity in tissue brain and liver (p <0.05), as compared to control, Figure
5.5.1.1. HgCl₂ treatment significantly depleted CAT activity as compared to control (p< 0.001). Recent study showed that HgCl₂ cause DNA damage and oxidative stress in human-derived liver cells and quercetin reduces these effects [Barcelos et al., 2011].

Figure 5.5.1.1

Cotreatment of onion extract, catechin and quercetin with HgCl₂ significantly (p< 0.01 & p< 0.05) improved the CAT activity as compared to HgCl₂ group. This finding is in agreement with the induction of CAT, SOD, and glutathione-S-transferases (GST) in HepG₂ cells by quercetin reported by Alia et al. [2006].

5.5.1.2 Superoxide Dismutase

SOD accelerates the dismutation of H₂O₂, also termed as a primary defence, as it prevents further generation of free radicals. It has been reported that the increased activity of SOD is known to serve as protective responses to eliminate reactive free radicals [Celik and Suzek, 2009]. In some studies, it has been indicated that superoxide radicals can inhibit CAT activity and the increased H₂O₂ resulting from CAT inhibition could finally inhibit SOD activity [Gultekin et al., 2000].
In this study, HgCl₂ treatment significantly depleted the SOD activity (p< 0.001), Figure 5.5.1.2. Co-treatment of onion extract, quercetin and catechin with HgCl₂ significantly (p< 0.01 & p< 0.05) improved the SOD activity as compared to HgCl₂ group. In general, higher SOD activity in experimental groups might offer some
protective effects against generation of free radicals, thus reducing the oxidative stress by enhancing the conversion of superoxide radicals to $H_2O_2$, followed by deactivation of $H_2O_2$ by GPx in $HgCl_2$ group [Heistad et al., 2009; Shakirin et al., 2012]. Our study is corroborated with the recent report according to which red onion may enhance antioxidant defense mechanism through the induction of plasma SOD and GPx activities and inhibited liver lipid peroxidation [Lee et al., 2012].

5.5.1.3 Intracellular Reduced Glutathione

GSH a tripeptide (L-r-glutamylcysteinyglycine) and a cysteine rich protein participates in the maintenance of cytoplasmic and membrane thiol status. It is an antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification of ROS. In the present investigation, $HgCl_2$ treatment significantly depleted GSH in liver and brain tissues as compared to control ($p<0.001$), Figure 5.5.1.3. The reduced level of hepatic GSH by $HgCl_2$ treatment could be probably due to either increased utilization of GSH by the cells act as scavengers of free radicals produced by $HgCl_2$ or increased utilization of GSH for the activity of GPx forming oxidized GSH (GSSG) due to increased generation of ROS. This is due to the fact that it has a great affinity for SH groups of biomolecules, thus depleting intracellular thiols including reduced glutathione [Hansen et al., 2006], due to which antioxidant GSH depletion by mercury may be a trigger for the production of reactive oxygen species (ROS) that induce lipid, protein, and DNA oxidation. Significant depletion of hepatic GSH has also been reported by Mohamed et al. [2010] in $HgCl_2$ intoxicated rats.

Treatment with onion extract and catechin caused slight increase in GSH level in liver and brain tissues ($p<0.05$), however, treatment with quercetin resulted in significantly higher GSH level as compared to control ($p<0.001$). Cotreatment of onion extract, catechin and quercetin with $HgCl_2$ significantly ($p<0.01$ & $p<0.001$) improved the GSH level as compared to $HgCl_2$ group. Significant enhancement and restoration of reduced GSH in rats treated with onion extracts and flavonoids definitely revealed the protective nature of the onion extract against $HgCl_2$ hepatotoxicity. This possible protective effect may be due to bioactive phytochemicals and nutrients in onion and their antioxidant and free radical scavenging activity [Griffiths et al., 2002, Ola-Mudathir et al., 2008].
5.5.1.4 Glutathione Peroxidases

Glutathione peroxidases (GPx) are antioxidant seleno enzymes that are present in the cytosol of cells or plasma; the kidney secretes GPx into plasma. The major function of these enzymes, which use glutathione as a substrate, is to reduce soluble hydrogen peroxide and alkyl peroxidases [Bebe and Panemangalore, 2003; Demir et al., 2011]. GPx converts hydrogen peroxide into H\textsubscript{2}O in the presence of oxidized glutathione [Kanbur et al., 2009]. Considering that glutathione- S-transferases are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to thiol group of GSH, producing less toxic forms [Mansour and Mossa, 2009]. Treatment with onion extract and catechin caused slight increase in GPx activity in tissue brain and liver (p < 0.05), however, treatment with quercetin resulted in significantly higher Gpx activity as compared to control (p< 0.001), Figure 5.5.1.4.

Figure 5.5.1.4

![Graph showing the effect of onion extract, catechin and quercetin on mercuric chloride (HgCl\textsubscript{2}) induced oxidative stress on enzyme glutathione peroxidase (GPx) activity in brain and liver of wistar strain rat. GPx activity is expressed as U / mg Protein. Values represent mean ±SD. *(p < 0.05) as compared to control. ** (p < 0.01) as compared to control. ♯ (p < 0.01) as compared to control, Onion+ HgCl\textsubscript{2}, Catechin+ HgCl\textsubscript{2} and Quercetin+ HgCl\textsubscript{2}.](image)

HgCl\textsubscript{2} treatment significantly depleted GPx as compared to control (p< 0.001). Inhibition of GPx activity is accompanied by a depletion of GSH, which may result in oxidative stress. Reduced GSH, in conjunction with GPx and glutathione-S-transferase (GST), is responsible for the GSH redox cycles that maintain the redox
status of tissues and protect structural and regulatory proteins against ROS-induced damage [Khan et al., 2007]. These components of Phase II metabolism catalyze the conjugation of electrophiles with glutathione and aid in their ultimate removal from the body. The decrease in GPx and GST activities can be seen to result directly from the decreased levels of GSH following exposure, since both enzymes depend on GSH for activity [Khan et al., 2007]. In the present study, the decreased GPx activity might reflect cellular oxidative stress due to HgCl₂ exposure. Notably, HgCl₂ can disrupt the activity of thiol- and selenol-containing proteins, such as Gpx, thioredoxin (Trx) and thioredoxin reductase (TrxR) [Carvalho et al., 2010; Branco et al., 2011]. These proteins are important components of the cellular antioxidant system, and their inhibition contributes to the disruption of the normal redox balance of brain cells [Farina et al., 2011].

Cotreatment of onion extract, catechin and quercetin with HgCl₂ significantly (p<0.01) improved the GPx activity as compared to HgCl₂ group. Even though it was not easy to explain the possible causes of this phenomenon, we explain that the tendency of increment of CAT and SOD activity and slight decrement of liver and brain MDA level resuled in a restoration of GPx activity.

### 5.5.2 Effect on Lipid peroxidation

MDA is the last product produced from lipid peroxidation created by ROS-induced damage to the cellular membrane. Oxidative stress is discussed as a possible cause of hepatocarcinogenesis in rodents and is suggested to result from excessive production of H₂O₂ from degradation of fatty acid. However, a previous report suggested that increased levels of the antioxidant glutathione can have a favorable effect on controlling the production of MDA [Karaoz et al., 2002]. HgCl₂ exerted a significant (p < 0.01) increase in brain and liver MDA level compared with the control whereas no statistically significant change in MDA level was observed in rats treated with either onion, catechin or quercetin, Figure 5.5.2. It has been demonstrated that mercury (II) decreases the antioxidative systems and produces oxidative damages via H₂O₂ generation thereby leading to lipid peroxidation [Jadhav et al., 2007]. All these possible mechanisms of HgCl₂ toxicity may lead to the formation of ROS, as found in
the present investigation. Therefore, an increase in the formation of ROS by HgCl\textsubscript{2} may induce membrane biochemical and functional alterations and thus induced liver cell damage.

**Figure 5.5.2**

Administration of onion, catechin or quercetin to HgCl\textsubscript{2}-intoxicated rats significantly (p < 0.01 & p < 0.001) reduced MDA level in the liver and brain. This result was in line with previous research indicating that onion extract lowered MDA levels in the testes of rats with cadmium toxicity and that MDA levels decreased significantly in rats with aflatoxin toxicity after the simultaneous consumption of garlic, onions, and cabbage [Ola-Mudathir et al., 2008; Abdel-Wahhab and Aly, 2003].

**5.5.3 Effect on Protein Oxidation**

**5.5.3.1 Protein**

The present results demonstrated that total protein was significantly decreased in liver and brain in response to HgCl\textsubscript{2} administered rats. The decreased level of total protein is concomitant with those observed by Thirumavukkarasu and Sakthisekaran [2003] and may be attributed to an increase in amino acids
deamination. In addition to the construction of cellular proteins, liver is the sole source of the bulk of the plasma proteins mainly albumin, fibrinogen, and prothrombin. Most of α and β globulins are also of hepatic origin [Sherlock and Dooley, 1993]. The lower protein levels that observed in HgCl₂ treated group (p < 0.001) might be also due to formation of the toxic compounds or reduction in the protein levels could be attributed to their damage by singlet oxygen, often due to oxidation of essential amino acids, Figure 5.5.3.1.

**Figure 5.5.3.1**

![Graph showing protein content in brain and liver](image)

**Figure 5.5.3.1. Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl₂) induced oxidative stress on protein content in brain and liver of wistar strain rat.** Protein content is expressed as mg / g tissue. Values represent mean ±SD. **(p < 0.001) as compared to HgCl₂.**(p < 0.01) as compared to Onion+ HgCl₂, Catechin+ HgCl₂ and Quercetin+ HgCl₂.

Further MDA formed during lipid peroxidation could react with –SH groups of proteins to damage them, thus inhibiting enzymes requiring –SH groups for their activities [Halliwell and Gutteridge, 1999]. The histochemical results of recent study revealed a decrease in glycogen, DNA and protein content in hepatocytes of HgCl₂ - treated rats. [Mahour and Saxena, 2007] also reported reduction in the amount of glycogen and protein in hepatic lobules after acute and sub-acute HgCl₂ intoxication. DNA was a vital molecule in the cell activities and was the main target for HgCl₂-induced cell injury [Schurz et al., 2000]. Cotreatment of onion extract, catechin and quercetin with HgCl₂ significantly restored the protein content of liver and brain near to the normal levels as compared to HgCl₂ group (p < 0.01).
5.5.3.2 Protein carbonyl

At the end of fourth week, PCO level was significantly increased in the HgCl$_2$ group compared with the control (p< 0.001), Figure 5.5.3.2. Several studies confirmed the decreased protein synthesis by mercury-intoxicated hepatocytes that were reflected by low serum albumin level and low protein content in hepatocytes concomitant with the ultrastructural changes noticed in rough endoplasmic reticulum. This may be attributed to the formation of mercaptides formed by interaction between mercury and intracellular thiol group of terminal cysteinyl residue of albumin molecule, and the formation of less stable complexes with other amino acid side chains [Mohamed et al., 2010]. ROS can convert proteins to carbonyl derivatives by a variety of oxidative mechanisms [Stadtman, 1992], and are considered sensitive indices of oxidative injury to proteins.

**Figure 5.5.3.2

The increase in PCO content was found to be correlated with decrease in protein content in HgCl$_2$ challenged rats. However, PCO level was significantly decreased in liver and brain at the end of the fourth week in the onion + HgCl$_2$, catechin + HgCl$_2$
group and quercetin + HgCl\textsubscript{2} group compared with the HgCl\textsubscript{2} group (p < 0.01 and p < 0.001). The results indicated that polyphenols are effective in preventing oxidative protein damages and restoring normal protein levels in tissues.

### 5.5.4 Effect on Plasma Membrane Redox System

Previous studies have provided evidence that oxidative stress and cellular energy deficits contribute to neuronal dysfunction and death in Alzheimer's disease [Parihar and Brewer, 2007]. The PMRS may play a particularly important role in protecting neurons against oxidative stress and energy impairment during aging because multiple PMRS enzymes are down-regulated in brain cells during normal aging in mice, and long-term dietary energy restriction in adult life preserves PMRS function and reduces oxidative damage to brain cells [Hyun et al., 2006a]. Elevated levels of PMRS in erythrocytes during state of oxidative stress is recently been highlighted [Rizvi et al., 2006]. A marked increase in PMRS activity in liver and brain was observed in HgCl\textsubscript{2} challenged rats as compared to control (p < 0.001), Figure 5.5.4. However, a significant decrease in the PMRS activity was observed in liver and brain of HgCl\textsubscript{2} challenged rats when supplemented with onion extract (p < 0.05), catechin or quercetin (p < 0.001) compared to HgCl\textsubscript{2} group, indicating that such treatment enhances antioxidant capacity and mitigates oxidative stress in liver and brain of HgCl\textsubscript{2} treated rats.

The variety of antioxidant phytochemicals in *Allium*, which protect against disease-causing oxidative damage, may act in single and combined fashion. Onions are rich in two chemical groups, two main components of onion, hydrophilic flavonoids (i.e. flavonol glucosides) and lipophilic organosulfur compounds (i.e. dipropyl sulfide and dipropyl trisulfide), are responsible for the antioxidant activity [Griffiths et al., 2002; Shon et al., 2004]. The phenolic hydroxyl groups in the structure of flavonoids have been recognized to function as electron or hydrogen donors, conferring a free radical scavenging effect [Shahidi et al., 1992]. Bioactive phytochemicals and nutrients in onion and garlic have been reported to exert antioxidant effects both in vitro and in vivo [Griffiths et al., 2002; Nuutila et al., 2003]. It is possible that the antioxidant
components of the extracts might act as sacrificial antioxidants, sparing the depletion of endogenous GSH, SOD and CAT occasioned by HgCl$_2$-induced oxidative stress. The ability of onion and garlic components to enhance SOD and CAT activities in carbon tetrachloride-induced toxicity has been demonstrated [El-Manakly et al. 1998]. Dok-Go et al. [2003] demonstrated that quercetin acts in many cell-free experimental systems to scavenge reactive oxygen radicals and to reduce oxidative DNA damage. In additions, quercetin is known to scavenge ROS generation in cardiomyocytes and thereby it protects against cell death in ischemia model. Previous in vivo studies indicate that quercetin decreases hepatic lipid peroxidation in rats [Choi et al., 2003; Duarte et al., 2001], decreases UV-light-induced increases in plasma MDA while increasing the levels of reduced glutathione [Kahraman and Inal, 2002], decreases plasma thiobarbituric acid reactive substances and hydroperoxides and restores the activities of SOD and CAT to near-normal levels in streptozotocin-induced diabetic rats [Mahesh and Menon, 2004].

Figure 5.5.4

Figure 5.5.4. Effect of onion extract, catechin and quercetin on mercuric chloride (HgCl$_2$) induced oxidative stress on plasma membrane redox system (PMRS) activity in brain and liver of wistar strain rat. PMRS activity is expressed as µmol/g tissue. Values represent mean ±SD. ** (p < 0.001) as compared to HgCl$_2$. *(p < 0.001) as compared to HgCl$_2$. ♯ (p < 0.05) as compared to HgCl$_2$. 
Catechin is known to suppress the formation of carcinogenic heterocyclic amines and nitrosamines. It binds to metal ions, preventing them from participating in peroxidase reactions. It also has the potential to scavenge reactive oxygen and nitrogen species, reducing their damage to lipid membranes, proteins, and nucleic acids in cell-free systems [Wiseman et al., 1997]. Catechin has shown protective effect on mammalian hepatic cells, leading to its therapeutic use in hepatitis [Blum et al., 1997]. The catechin incorporated in cell membranes was shown to prevent or reduce the morphological and biochemical alterations of hepatocytes induced by xenobiotics [Rosen, 2012].

5.6 EFFECT OF DIFFERENT LAYERS OF ONION EXTRACT ON OXIDATIVE STRESS BIOMARKERS IN ALLOXAN INDUCED DIABETIC RATS IN VIVO

Excess generation of highly reactive free radicals (largely due to hyperglycemia) or impaired antioxidant defenses results in increased oxidative stress by a mechanism involving glucose oxidation followed by protein glycation and oxidative degeneration [Ceriello 2000; Halliwell and Gutteridge, 1990]. Increased oxidative stress further exacerbates the development and progression of diabetes and its complications through various mechanisms, including activation of transcription factors, advanced glycated end products (AGEs), and protein kinase C [Maritim et al., 2003]. In the present work, involvement of free radicals in progression of disease and protective effects of different layers of onion extracts (OLE and ILE) in alloxan-induced diabetic rats by testing its effect on biomarkers of oxidative stress, along with antihyperlipidemic and antioxidant activities has been examined. The diabetogenic agent alloxan, a chemically unstable pyrimidine derivative, is cytotoxic to insulin-producing pancreatic β-cells, resulting in reduction in the synthesis and the release of insulin due to excessive production of ROS. Alloxan inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the β-cell, and causes a state of insulin-dependent diabetes due to its ability to generate oxygen free radicals [Szkudelski, 2001].
5.6.1 Effect of OLE, ILE and Quercetin on Blood Glucose Levels

It is well documented that antidiabetic drugs treat diabetes mellitus by lowering glucose levels in the blood. The changes in blood glucose level in all five groups of animals are given in Table 1. Blood glucose levels of diabetic control rats were significantly (p < 0.05) higher than those in normal rats. Onion extracts (both OLE and ILE) as well as quercetin supplementation resulted in a significant (p < 0.05) decrease in blood glucose levels of diabetic treated group.

In our study, we have found that 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. This might be due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or a decrease in the intestinal absorption of glucose [Malviya et al., 2010]. Onion extracts may have worked through this mechanism or by stimulation of surviving beta cells to release more insulin just like glibenclamide [Ivorra et al., 1988].

5.6.2 Serum Free Fatty Acid

Diabetes is also associated with hyperlipidemia. Insulin resistance in adipose tissue is characterized by excessive rates of lipolysis, increased plasma FFA levels despite hyperinsulinemia, and impaired suppression of plasma FFA levels by insulin [McGarry, 2002]. Figure 5.6.1 shows the level of serum free fatty acid with serum free fatty acid significantly (p < 0.001) higher in diabetic rats as compared to normal rats. Onion extracts (both OLE and ILE) as well as quercetin supplementation lowered the serum free fatty acid as compared to untreated diabetic rats (p < 0.05).
Table 1. Effect of onion outer layer extract (OLE), onion inner layer extract (ILE), quercetin and insulin on blood glucose levels of alloxan induced diabetic rats in vivo

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>OLE</th>
<th>ILE</th>
<th>Quercetin</th>
<th>Diabetic Control</th>
<th>Diabetic + OLE</th>
<th>Diabetic + ILE</th>
<th>Diabetic + Quercetin</th>
<th>Diabetic + Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>87.3 ±7.2</td>
<td>85.6±8.6</td>
<td>93.6±4.9</td>
<td>90.2±8.2</td>
<td>270.3±9.5*</td>
<td>272.3±10.1</td>
<td>269.0±7.5</td>
<td>273.3±11.0</td>
<td>276.6±10.0</td>
</tr>
<tr>
<td>7th day</td>
<td>88.3 ± 8.7</td>
<td>87.6±7.0</td>
<td>85.0±3.0</td>
<td>83.0±7.5</td>
<td>280.3±10.1*</td>
<td>186.6±7.6**</td>
<td>193.3±4.5**</td>
<td>183.6±5.0**</td>
<td>228.3±9.3**</td>
</tr>
<tr>
<td>14th day</td>
<td>86.3 ± 3.7</td>
<td>89.6±7.4</td>
<td>87.6±7.4</td>
<td>87.1±6.2</td>
<td>293.0±4.3*</td>
<td>164.0±9.5**</td>
<td>169.0±6.0**</td>
<td>158.6±4.0**</td>
<td>175.0±7.2**</td>
</tr>
<tr>
<td>21st day</td>
<td>88.6 ± 5.6</td>
<td>85.6±5.7</td>
<td>91.6±5.7</td>
<td>85.1±6.4</td>
<td>316.0±7.3*</td>
<td>124.0±7.8**</td>
<td>131.0±6.2**</td>
<td>124.3±8.0**</td>
<td>138.3±8.5**</td>
</tr>
<tr>
<td>28th day</td>
<td>90.3 ± 4.5</td>
<td>92.3±8.9</td>
<td>84.6±4.0</td>
<td>91.2±5.9</td>
<td>332.3±11.2*</td>
<td>104.0±9.5**</td>
<td>110.0±11.0**</td>
<td>101.6±9.4**</td>
<td>95.6±9.4**</td>
</tr>
</tbody>
</table>

Values represent mean ±SD.

*p < 0.01 Diabetic group as compared to control group.

**p < 0.05 Experimental group as compared to Diabetic control.
Table 2. Effect of onion outer layer extract (OLE), onion inner layer extract (ILE), quercetin and insulin on lipid profile of alloxan induced diabetic rats *in vivo*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>OLE</th>
<th>ILE</th>
<th>Quercetin</th>
<th>Diabetic Control</th>
<th>Diabetic + OLE</th>
<th>Diabetic + ILE</th>
<th>Diabetic + Quercetin</th>
<th>Diabetic + Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>68.3±7.0</td>
<td>64.7±7.1</td>
<td>63.5±6</td>
<td>69.7±4.7</td>
<td>105.6±7.1**a</td>
<td>84.2±3.5**ab</td>
<td>91.7±6.0</td>
<td>82.7±5.7**ab</td>
<td>71.5±3.3**ab</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>75.5±3.8</td>
<td>77.3±3.8</td>
<td>78.7±9.7</td>
<td>62.7±15.0</td>
<td>156.6±7.7**a</td>
<td>101.8±20.5**ab</td>
<td>128.9±14.1**b</td>
<td>88.3±7.5**b</td>
<td>91.8±13.2**ab</td>
</tr>
<tr>
<td>HDL</td>
<td>28.6±2.8</td>
<td>26.3±4.9</td>
<td>28.9±4.5</td>
<td>33.8±2.6</td>
<td>23.7±1.1 **a</td>
<td>28.8±2.7**b</td>
<td>26.0±0.7**h</td>
<td>29.7±3.1**h</td>
<td>26.4±4.0</td>
</tr>
<tr>
<td>LDL</td>
<td>24.7±3.5</td>
<td>22.8±1.4</td>
<td>18.9±0.7</td>
<td>23.3±0.9</td>
<td>50.7±2.6**a</td>
<td>35.0±2.9**ab</td>
<td>39.8±0.9**ab</td>
<td>34.6±0.5**ab</td>
<td>27.9±3.7**ab</td>
</tr>
<tr>
<td>VLDL</td>
<td>15.1±0.8</td>
<td>15.5±0.8</td>
<td>15.7±1.9</td>
<td>12.5±4.1</td>
<td>31.3±1.5**a</td>
<td>20.4±4.1**ab</td>
<td>25.8±2.8**b</td>
<td>18.3±2.6**ab</td>
<td>17.1±3.0**ab</td>
</tr>
</tbody>
</table>

Values represent mean ±SD. *p < 0.05; **p < 0.01.

*a* Diabetic group as compared to control group.

*b* Experimental group as compared to Diabetic control.
RESULTS AND DISCUSSION

Figure 5.6.1

![Figure 5.6.1.](image)

**Figure 5.6.1.** Effect of onion outer layer extract (OLE), onion inner layer extract (ILE), quercetin and insulin on alloxan induced diabetic rats on serum free fatty acid (FFA) *in vivo*. FFA levels is expressed as μM. Values represent mean ±SD. *(p < 0.001)* as compared to control. ** *(p < 0.05)* as compared to Diabetic.

Figure 5.6.2

![Figure 5.6.2](image)

**Figure 5.6.2.** Effect of onion outer layer extract (OLE), onion inner layer extract (ILE), quercetin and insulin on alloxan induced diabetic rats on total antioxidant capacity of plasma *(measured in terms of FRAP value in vivo)*. FRAP value is expressed as μmol Fe (II)/L of plasma. Values represent mean ±SD. ♯ *(p < 0.01)* as compared to control * *(p < 0.001)* as compared to control. ** *(p < 0.05)* as compared to Diabetic+OLE.

5.6.3 Serum Lipid Profile

Elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism. The insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein
RESULTS AND DISCUSSION

metabolism during diabetes [Ranganathan et al., 2000]. The lipoprotein levels in the alloxan induced diabetic rats of the present study reveal a significant alter in lipoprotein metabolism. The serum total cholesterol content increased significantly in diabetic animals. The elevated hypertriglyceridemia was increased in the synthesis of triglyceride rich lipoprotein particles in liver diminished catabolism in diabetic rats. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver [Ohno, 2000]. Table 2 describes the effect of extracts on serum lipid profile. An increase in serum total cholesterol, triglyceride, LDL, VLDL levels, with reduction in HDL levels was observed in alloxan induced diabetic rats when compared with the normal control rats. Onion extract supplementation (both OLE and ILE) offered a significant (p < 0.05) decrease in the serum triglycerides, total cholesterol, LDL and VLDL levels, and an increase in the HDL cholesterol levels.

The increased levels of LDL in the diabetic animals might be due to over production of LDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx [Coppack, 1994]. HDL was significantly reduced in the diabetic rats which indicate a positive risk factor for atherosclerosis. After the administration of onion extracts to the alloxan induced diabetic rats revealed a decreased serum TG, TC, LDL-c and VLDL-c levels. These effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis that are under the control of insulin [Sharma et al., 2003]. This extract (OLE and ILE) supplementation also resulted in significant attenuation in the level HDLc in serum towards the control level, which again strengthens the hypolipidemic effect of this extract.

5.6.4 Plasma Antioxidant Capacity by FRAP Assay

Increased oxidative stress due to persistent and chronic hyperglycaemia depletes the activity of antioxidative defense system that further promotes de novo free radicals generation. This can further lead to damage of cellular organelles and enzymes, and development of insulin resistance. All the groups were studied for their antioxidant activity by FRAP assay, Figure 5.6.2. Treatment with quercetin improved the antioxidant activities (p< 0.01), however, the antioxidant activities were significantly
(p< 0.001) reduced in rats treated with alloxan as compared to normal control rats. Significantly (p< 0.05) higher antioxidant activity of OLE was observed as compared to ILE upon supplementation to diabetic rats. Quercetin showed highest improvement in the antioxidant potential of the plasma compared to diabetic treated group (p< 0.001).

5.6.5 Erythrocyte Lipid Peroxidation

Erythrocyte MDA level was significantly increased in the alloxan induced diabetic rats as compared with normal control rats (p< 0.001), and, in contrast, MDA level was significantly decreased in erythrocytes when supplemented with onion extracts (both OLE and ILE) (p< 0.01), with higher protection by OLE as compared to ILE (p< 0.05), Figure 5.6.3. Diabetic rats treated with insulin as well as quercetin restored the MDA level near to normal control rats. Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors [Arulselvan and Subramanian, 2007]. In our study MDA levels in diabetes group were found to be higher than those in control group, indicating increased free radical generation.

However, supplementation of onion extracts (OLE and ILE) caused marked reduction in lipid peroxidation and antioxidative activity of plasma. Our results are in agreement with the previous reports which show that supplementation of onion rich diet results in increase in antioxidative ability of rat plasma [Azuma et al., 2007]. This may be due to the presence of flavonoids (especially quercetin). Quercetin is known to exert multiple mechanisms including antioxidant activity, antiinflammation, modification of signal transduction pathways, and interactions with receptors and other proteins. Recent studies have shown that quercetin could ameliorate diabetes-induced oxidative stress and preserved pancreatic beta cell integrity [Rizvi and Mishra, 2009; Oršolic et al., 2011; Kanter et al., 2012]. It could enhance adiponectin secretion by a PPAR-c independent mechanism, prevent impairment of insulin sensitivity without affecting body weight and composition [Wein et al., 2010], inhibit hepatic stellate cells (HSC) activation [Zois et al., 2008], and reduce the expression of human C-reactive protein and cardiovascular risk factors (such as undergoing apoptosis and fibrinogen) in mice.
Figure 5.6.3

Figure 5.6.3. Effect of onion outer layer extract (OLE), onion inner layer extract (ILE), quercetin and insulin on alloxan induced diabetic rats on erythrocyte malondialdehyde (MDA) content in vivo. Concentration of MDA is expressed as nmol/mL of packed RBC. Values represent mean ±SD. ***(p < 0.001) as compared to diabetic. **(p < 0.01) as compared to Diabetic+OLE, Diabetic+ILE, Diabetic+Quercetin, Diabetic+Insulin. *(p < 0.05) as compared to Diabetic+ILE.

Figure 5.6.4

Figure 5.6.4. Effect of onion outer layer extract (OLE), onion inner layer extract (ILE), quercetin and insulin on alloxan induced diabetic rats on erythrocyte reduced glutathione (GSH) in vivo. Concentration of GSH is expressed as mg/mL PRBC. Values represent mean ±SD. ***(p < 0.001) as compared to diabetic. **(p < 0.01) as compared to Diabetic+OLE, Diabetic+ILE and Diabetic+Quercetin. *(p < 0.05) as compared to Diabetic+ILE.

5.6.6 Intracellular Reduced Glutathione

Glutathione, present in millimolar concentrations including erythrocytes maintains the intracellular redox balance and to eliminate ROS. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases. Alloxan treatment significantly depleted erythrocyte GSH levels as compared to normal control rats (p< 0.001), however, treatment with onion extract
(both OLE and ILE) and quercetin caused a significant increase in GSH level (p <0.05), with OLE being more effective as compared to ILE supplementation, Figure 5.6.4. In the present study, a significant depletion of erythrocyte GSH in alloxan-induced diabetic rats demonstrates that a condition of oxidative stress prevails thereby disrupting the actions of antioxidant enzymes.

It is possible that the antioxidant components of the extracts might act as sacrificial antioxidants sparing the depletion of endogenous GSH during diabetic-induced oxidative stress. Furthermore, dietary polyphenols have been shown to upregulate the expression of c-glutamylcysteine synthetase, the rate-limiting enzyme in the biosynthesis of GSH [Moskaug et al., 2005]. This may explain, in part, the enhancement of GSH in alloxan induced diabetic rats treated with onion extracts.

5.6.7 Plasma Sialic Acid

Plasma sialic acid level was significantly increased in the alloxan induced diabetic rats as compared with normal control rats (p< 0.001), and, in contrast, treatment with insulin, quercetin or onion extracts (OLE & ILE) restored cellular sialic acid with decreased plasma sialic content to the same levels as in control (p< 0.01 & p< 0.001), Figure 5.6.5.

Diabetic induced oxidative stress, resulted in modifications of erythrocyte membrane thereby causing increased levels of plasma sialic acid due to detachment or degradation of sialic acid from the erythrocyte membrane, causing changes in cell function or activity. The plasma sialic acid level has also been considered as an important parameter in identifying subjects prone to develop type-2 diabetes [Gavella et al., 2003]. It has been shown previously that desialylation of adipocytes and hepatocytes leads to a reduction in insulin action associated with insulin resistance, whereas removal of sialic acid from hepatocytes reduces insulin-stimulated lipogenesis [Peppa et al., 2008]. The role of onion extract in protecting against diabetes has been reported previously [Azuma et al., 2007] and a decrease in plasma sialic acid shown in this study is the novel finding as an indicative of a decrease progression of diabetic complications.
It should be noted that OLE showed higher antioxidant activity and GSH content as compared to ILE. This could be explained by the fact that there might be differences in the distribution of flavonol mainly QDG and QMG in different living layers of onion with higher flavonoid content of outer living layers as compared to outer dry scale and inner layers [Beesk et al., 2010]. It can be concluded from the above study that different layers of onion have confirmed the hypoglycemic activities. 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.

Figure 5.6.5

Figure 5.6.5. Effect of onion outer layer extract (OLE), onion inner layer extract (ILE), quercetin and insulin on alloxan induced diabetic rats on plasma sialic acid level in vivo. Concentration of plasma sialic acid is expressed as µM. Values represent mean ±SD. **(p < 0.001) as compared to diabetic. *(p < 0.01) as compared to Diabetic+OLE, Diabetic+ILE and Diabetic+Insulin. ##(p < 0.001) as compared to Diabetic.