CHAPTER 3

OBJECTIVES OF THE STUDY
In recent years, there is renewed interest towards study of plants and their isolated compounds for the prevention of diseases and diverse pathological conditions by offering protection against cellular damage and oxidative stress [Hassan et al., 2010]. Researches on traditional medicinal plants are gradually emerging as viable alternatives to conventional drugs for various free radical mediated diseases. Because of their many intriguing biological effects, flavonoids, a class of polyphenolic compounds widely distributed in plants, received special attention as dietary constituents during the last few years [Del Rio et al., 2010].

Onion one of the oldest cultivated plants used for culinary, medicinal and spiritual purposes, recently gaining attention for its multiple functional properties which include free radical scavenging activity, immune stimulation, cardioprotective effects, anti-cancer and anti-infectious property [Desjardins, 2008]. The phytochemical composition of onions is believed to vary according to species and cultivation technique. Among the species of onions, the red onion is abundant in polyphenols, flavonoids, flavonol, and tannin [Gorinstein et al., 2010]. Some researchers reported that red onions had quercetin levels that were 14-fold that of garlic and levels that were twofold that of white onions [Gorinstein et al., 2008]. Additionally, onion peel contains flavonoid levels that are 48-fold of that in its flesh, and cell-based investigations found that the peel has a greater capacity for controlling lipid peroxidation than the flesh does [Jang and Im, 2009]. In a study of the antioxidant effects of onion flesh and peel, rats that consumed the peel had higher levels of antioxidants and lower levels of lipid peroxide than rats that ate the flesh did [Park and Kim, 2005; Park et al., 2007]. Previous studies show significant differences in the levels of QDG and QMG between different onion cultivars [Price and Rhodes, 1997; Slimestad et al., 2007; Bonaccorsi et al., 2008], alongwith it differences in the flavonoid content were also found in between different scales of onion [Beesk et al., 2010; Bilyk et al., 1984; Prakash et al., 2007], with the outer dry peel having greater capacity for controlling lipid peroxidation than the flesh does [Jang and Im, 2009]. Especially higher concentrations of quercetin occur in the outer dry layers of onion bulb [Smith et al., 2003].

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

The objectives of the study are:

a. To evaluate the nutritional properties of common *Allium* species used as vegetables/spices in North India. The parameters include: nutritive value, fibre content, polyphenol content, trace element content and other important chemical constituents & elemental analysis


c. Relating the nutritive and biochemical properties of *Allium* species to their medicinal effects.

**Detail of the parameters studied to accomplish the objectives enumerated above:**

1. **Antioxidant Activity of Different Layers of Onion Extract at Two Different Stages of Maturation *In vitro***

   i. Determination of Total Phenolic Content
   
   ii. Determination of Total Flavonoid Content
   
   iii. Determination of Antioxidant activity by FRAP assay
   
   iv. Determination of Free Radical Scavenging Activity using DPPH Radical
   
   v. Determination of Percent Scavenging Activity of Hydrogen Peroxide
   
   vi. Determination of Reducing Power Activity
   
   vii. Determination of Hydroxyl Radical Scavenging Activity
viii. Determination of Metal Chelating Activity
ix. Determination of α-Amylase Inhibitory Activity

2. Proximate Composition of Different Layers of Onion Extract at Two Different Stages of Maturation

i. Determination of Moisture Content
ii. Determination of Protein Content
iii. Determination of Total Ash Content
iv. Determination of Crude Fibre Content
v. Determination of Fat Content
vi. Determination of Carbohydrate Content

3. Effect of Different Layers of Onion Extract on Oxidative Stress Biomarkers in Erythrocytes subjected to Oxidative Stress by tBHP

In vitro: Comparison with Quercetin

i. Assessment of Percent Oxidative Hemolysis
ii. Assessment of Erythrocyte Lipid peroxidation
iii. Assessment of Erythrocyte Glutathione content
iv. Assessment of Erythrocyte Plasma Membrane Redox System

4. Effect of Onion Extract on Oxidative Stress Biomarkers in Erythrocytes subjected to Oxidative Stress by Mercuric Chloride

In vivo: Comparison with Quercetin and Catechin

i. Effect of Mercuric Chloride Treatment on Body Weight of Rats in a 30 day period
ii. Effect on Plasma Antioxidant Capacity
   a. Plasma Antioxidant Capacity by Frap Assay
   b. Plasma Radical Scavenging Activity (by using DPPH Radical)
iii. Effect on Erythrocyte and Plasma Lipid Oxidation
iv. Effect on Plasma Low-Density Lipoprotein Oxidation
v. Effect on Protein Oxidation
   a. Protein Carbonyl
OBJECTIVES OF THE STUDY

b. Advanced Oxidation Protein Products
c. Protein Hydroperoxides
d. Total Thiol

vi. Effect on Plasma Sialic Acid
vii. Effect on Intracellular Reduced Glutathione
viii. Effect on Plasma Ascorbic Acid
ix. Effect on Plasma Membrane Redox System
x. Effect on Antioxidant Enzyme Paraoxonase 1
xi. Effect on Acetylcholinesterase Activity

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

i. Effect on Enzymatic and Non Enzymatic Antioxidants
   a. Catalase
   b. Superoxide Dismutase
   c. Intracellular Reduced Glutathione
   d. Glutathione Peroxidase

ii. Effect on Lipid Peroxidation

iii. Effect on Protein Oxidation
   a. Protein
   b. Protein Carbonyl

iv. Effect on Plasma Membrane Redox System

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

i. Effect of Blood Glucose Levels
ii. Effect on Serum Free Fatty Acid
iii. Effect on Serum Lipid Profile
iv. Effect on Plasma Antioxidant Capacity by Frap Assay
v. Effect on Erythrocyte Lipid Peroxidation
vi. Effect on Erythrocyte Reduced Glutathione
vii. Effect on Plasma Sialic Acid Levels