DISSUSSION
Lipid peroxidation, which involves a series of free radical mediated chain reaction processes, is also associated with several types of biological damages like atherosclerosis, cancer, cataract, rheumatoid arthritis and other neurodegenerative diseases (Niki et al., 2005; Nascimento et al., 2005, Arora et al., 2002, Gulcin et al., 2005). Therefore much attention has been focused on the use of antioxidants, especially natural antioxidants. These antioxidants constitute a range of substances that play a role in protecting biological systems against the deleterious effects of oxidative processes and macromolecules, such as proteins, lipids, carbohydrates and DNA (Halliwell and Gutteridge, 1989). These antioxidants will inhibit lipid peroxidation and protect from damages due to free radicals.

The stimulation of peroxidation is believed to be dependent on generated Fe$^{2+}$, free radicals like hydroxyl radicals (OH), singlet oxygen (O$_2$), and peroxyl radical (Chung et al., 2006; Gulcin et al., 2005).

In the present investigation the model system (pea seedlings) of 5 days was treated with different concentrations (1 to 10 mM) of FeSO$_4$, and TBARS values were analyzed. Maximum TBARS was detected in the 10 mM FeSO$_4$ treated seedlings and followed by other concentrations when compared to control. The results point out that FeSO$_4$ promotes TBARS production (Table 1). From the result it is evident that the transition metal Fe$^{2+}$ plays a key role in the initiation of lipid peroxidation. Similar reports have been observed with similar transition metal salts (Chung et al., 2006, Halliwell and Gutteridege, 1999 and Dixit et al., 2001).
In the presence of reducing compounds such as Fe$^{+2}$, which have sufficient affinity to oxygen, superoxide dismutase is formed from oxygen. This superoxide dismutase spontaneously reacts with hydrogen to form hydrogen peroxide.

$$O_2 + O_2 + 2H^+ \rightarrow H_2O_2 + O_2$$

Fe$^{+2}$ reduces H$_2$O$_2$ yielding highly reactive hydroxyl radical OH by the way of Fenton reaction

$$H_2O_2 + Fe^{+2} \rightarrow Fe^{+3} + OH^- + OH^-$$

Fe$^{+2}$ catalyzed production of extremely reactive oxygen species seems to play a central role in general pathology (Hippeli et al., 1999) and membrane lipid damage both in vivo and in vitro (Ohyashiki et al., 1995, Ohyashiki et al 1998, Ramanarayan et al., 2000, Yamamoto et al., 2001 and Shalata and Neuman, 2001). Reactive oxygen intermediate is also implicated in cognate redox signaling in disease resistance (Hippeli et al., 1999; Grant and Loake, 2000).

Iron is the most abundant and important catalyst in ROS production. Therefore its metabolism is under the light control in plants and animals (Hippeli et al., 1999). Thus, the lipid peroxidation level is directly related to Fe$^{+2}$ concentrations.

Several investigations have suggested that the iron-catalyzed decomposition of lipid hydroperoxides (LOOH) is the primary driving force of Fe$^{+2}$ initiated lipid peroxidation. In addition, that Fe$^{+2}$ enhances the Fe$^{+2}$ catalyzed LOOH dependent lipid peroxidation and the oxidation of Fe$^{+2}$ by LOOH results in the formation of some iron species. These findings suggested
that initial rate of Fe$^{+2}$ oxidation may be affected by the presence of LOOH in lipid preparations. On the contrary some of the workers state that cadmium enhances lipid peroxidation and increases the concentrations of H$_2$O$_2$. It may due to ROS generation (Dixit et al., 2001). Peroxidation can be initiated by different compounds like azo initiators, photo sensitizers, transition metals like Fe$^{+2}$ and light. This initiator generates singlet oxygen or free radicals, which would in turn trigger peroxidation chain reaction (Ramanarayan et al., 2000). Lipid peroxidation is inhibited by naturally occurring α-tocopherol, β-carotene and other carotenoids (Halliwell and Gutteridege., 1985).

In order to check the natural product antioxidant property the plant Allophyllus cobbe L was selected. A. cobbe plant was used in many ayurvedic medicine mainly used for tapeworms, elephantiasis and colic diseases (Das and Kumar, 2003) and in Western Ghats herbal healers use oil extract of A. cobbe for burns and as wound healing medicine (Harsha, 2004). Therefore, the A. cobbe leaf extract was used to investigate its antioxidant potential. The leaf extracts of oil, acetone and chloroform was treated to 5days old pea seedlings to check lipid peroxidation inhibition activity (antioxidant property).

In the present investigation oil extract of A. cobbe was treated with and without combination of Fe$^{+2}$. Result point out that A. cobbe oil extract shows higher (34.11 μg/M) TBARS value than control (24.54μg/M) (Table 3) and with the combination of Fe$^{+2}$ it showed still higher (85.85μg/M) TBARS value in the roots (Fig 3). According to Grant and Loake (2000) plants produce oxidative burst following recognition of pathogen. This is necessary for programmed cell death and possibly to induce peroxidative damage to pathogenic bacteria. In this content it is possible that the oil extract of A. cobbe...
appear as a pro-oxidant with effect on membrane and peroxidative damage to membrane lipids (Fig 3).

Similarly in the present investigation, the acetone extracts of *A. cobbe* was tested to check its efficacy on lipid peroxidation, on pea roots, in combination of Fe$^{2+}$ and without Fe$^{2+}$. All the three concentrations (100µg, 250µg and 500µg) of acetone extract showed inhibitory action on roots (Table 4, Fig 4). In the similar way chloroform extract of *A. cobbe* of similar concentrations were treated on roots. Here also all extracts showed antioxidant property on Fe$^{2+}$ treated and non treated seedlings (Table 5, Fig 5).

The highest inhibition of lipid peroxidation was observed in chloroform extract compared to control but there was no significant inhibition of lipid peroxidation of oil extract (Table 3, Fig 3). But the acetone extract showed more or less same inhibition of lipid peroxidation at all the three tested concentrations (Table 4, Fig 4). Further chloroform extract showed highest inhibition of lipid peroxidation (19.03 µg/M) at 500 µg concentration (Table 5, Fig 5). Hence it clearly suggests that the chloroform extract has more Fe chelating ability when compared to acetone extract. Plants have almost limitless ability to synthesize aromatic substances that have been evaluated for their therapeutic potential. These include alkaloids, coumarins, saponins and flavonoids. Flavanoids are probably the best known of these substances due to their antioxidant properties. The therapeutic benefits of small plant species used by traditional herbalists, at least in part, was attributed to their effective inhibition of oxidative processes. Several of these herbal medicines are used traditionally in treating liver diseases, where *Pistacia lentiscus* was found to be effective in suppressing iron induced lipid peroxidation in rat
homogenates. In Trolox, the water soluble analog of vitamin E, did not adversely effect cell membranes integrity or suppress mitochondrial respiration in cultured HEPG2 and PG12 cells. The earlier reports also suggest that chelating agent are secondary antioxidants because they reduce redox potential there by stabilizing the oxidized form of metal ion. The results obtained clearly demonstrated that chloroform extract has marked capacity of metal chelating ability, suggesting their action on peroxidation protector.

For further investigation, ascorbic acid was used as standard antioxidant. Ascorbic acid (vitamin C) is water-soluble antioxidant molecule which acts as a primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide. In addition it acts directly to neutralize superoxide radicals during reductive recycling of oxidized form of α-tocopherol (Shalata and Neuman, 2001). Standard ascorbic acid (0.5mM) was treated to the pea seedlings with FeSO₄ as well as without FeSO₄. It was observed that there was increase in the inhibition of lipid peoxidation when compared with control (Table13). The extracts and standard ascorbic acid have the metal chelating ability, which helps in the prevention of lipid peroxidation.

An attempt was also made to investigate the antioxidant activity of standard ascorbic acid along with salicylic acid (SA). SA first discovered from salix plant (Willow) as a major component in the extracts from willow tree bark that had been used as a natural anti-inflammatory drug from the ancient time to the 18th century (Rainsford, 1984; Weissman 1991). Salicylic acid is an endogenous growth regulator of phenolic nature, which participates in regulation of physiological purposes in plants, it also plays a role in natural indicator of theromogenesis in Arium lily. It also induces flowering in range of plants (Sakhabutdinova et al., 2003) controls uptake by roots and stomatal
conductivity (Raskin, 1992). Further salicylic acid is a natural signal molecule which plays an important role in regulating a number of physiological processes in plants (Janda et al. 1999; Mishra and Choudhuri 1999; Singh and Usha, 2003). It has been known for many years that exogenous salicylic acid is involved in the defense against pathogen attack and more recently its role has been widely investigated in both biotic and a biotic stresses. Previous studies have also shown that SA could ameliorate the damaging agents of heavy metals as membrane in rice (Mishra and Choudhuri, 1999) and also involved in regulation of antioxidant enzymes and lipid peroxidation during heat stress.

Present investigation interestingly revealed that the SA increases lipid peroxidation activity in the model system, but there was inhibition of lipid peroxidation along with ascorbic acid. Thus it clearly suggests that the earlier report of exogenous SA is not reduced by another antioxidant present in model system leads to pro-oxidation process (Tiwari, 2000). Here it was observed that, in plant extract the excess α-tocopherol becomes pro-oxidant and not reduced. Hence the present investigation reveals that ascorbic acid has shown potential antioxidant property as reported by the earlier workers.

Natural products play key role in most of therapeutic properties. Most of these natural products have been derived from plants and successfully proved to have promising and high therapeutic values. Honey is also considered as one of the natural products having many therapeutic values. Honey has a long history of use in our medicinal systems. It was used by the ancient Greeks and Sumerians (Molan, 1995). In ancient Egypt it was used for wound treatment, mixed with grease and fibre. Hippocrates recommended honey and vinegar for pain and water and honey for thirst and a mixture of honey, to treat acute ulcers (Beck and Smedley, 1994; Zumla and Lulat, 1989). The bible maintains the use
of honey in eye problem (Beck and Smedly, 1994). In ayurvedic medicine, honey is described as the nectar of legend and its use is recommended for various conditions (Subrahmanyam, 1996).

Honey is a popular sweetener and common household product used throughout the world. Human use of honey is traced to approximately 800 yrs ago as depicted by Stone Age painting. The latter described the use of honey in baldness, contraception, wound healing, laxative action, cough and sore throat, eye disease, topical antiseptics, prevention and treatment of scars. In the twentieth century, antimicrobial and wound healing properties of honey have been reported.

It also contains proteins, vitamins and minerals. Honey has good quantities of antioxidants both with enzymatic and non-enzymatic activities. Depending upon the source varying concentrations of catalase, flavonoids, ascorbic acid and alkoloids are present. In general, the darker the honey, more is the antioxidant activity. Hence keeping all this in mind, honey was also subjected for investigation. Honey was treated to the pea seedlings along with FeSO₄ and SA, where honey proved to be as one of the most promising antioxidants with an increase in the inhibition of lipid peroxidation as compared to control in all the combinations. Honey, as the extracts of *A. cobbe*, also had the ability of metal chelating and hence acts as peroxidation protector. Hence, in the further days honey can be explored for the further investigation of all parameters of antioxidants.
In conclusion, this study demonstrates that the extracts from *A. cobbe* and honey are safe in antioxidant activity. The extracts had radical scavenger effects, reductive capability for reducing Fe^{3+} to Fe^{2+} and anti-lipid peroxidation activity in pea root system. These extracts of *A. cobbe* and honey significantly protect against oxidative damage induced by various oxidants. The antioxidant activity of *A. cobbe* extract related here brings basis of the traditional use of plant in different health problems especially for those involving the complex inflammatory process in which ROS free radicals play determinant role. The high level of activity measured for the extract is strongly supporting the beneficial role of herbal extracts in phyto-therapeutic preparations and encouraging the isolation and identification of the active constituents.

These results may shed light on an important biological homeostasis involving pro-oxidant and antioxidant profiles formulations. They may have significant potential applications in food industry - for preservation of food products and advantages over synthetic food antioxidants.

Further studies are needed to evaluate the *in vivo* antioxidant potential of these extracts in animal models and isolate the antioxidative components in *A. cobbe* and honey.