CONCLUSION

Careful discussion of the findings of the present study indicates towards following facts

(1) Effect of cadmium is of long duration because elevated or decreased levels of different parameters remained so even when cadmium feeding was discontinued up to 15 days. This fact is already well established and well documented. Another finding is that cadmium affects all organs of the body, some organs are more severely affected and others are less affected. Liver, kidney and testes are organs which show highly significant changes in various parameters studied and on the other hand muscles and brain show less significant changes in the parameters studied.

Cadmium is an accumulative metal and tissue concentration of cadmium is known to increase up to the ages of 50 to 60 years (Friberg et. al.1974, 1986), therefore all of us are in high risk groups with the metal accumulated in tissues.

(2) Effect of C. sinensis is dose dependent, as also reported by Yung-His-Kao and co-workers (2000). In almost all parameters 2 mg dose of C. sinensis caused some positive changes but in some rats some biochemical parameters were normal and in others remained increased or decreased accordingly. 4 mg dose of C. sinensis was able to protect the tissues against the damage caused by co-treatment with cadmium, hence the normal biochemical parameters were observed in most of the experimental animals.

(3) Various enzymes such as alkaline phosphatase, acid phosphatase, alanine transminase (ALT), asparate transaminase (AST) are originally found in cytoplasm of the cells of body organs as these enzymes play important role in metabolism. The level of these enzymes varies according to the organ concerned. For example, concentration of alkaline phosphatase, acid phosphatase, alanine transminase (ALT), asparate transaminase (AST) are very high in liver and kidney
tissue but quite low in other tissues like brain, muscles etc. It is but natural because liver is the seat of metabolism in the body. During the present study increase or decrease in the levels of above mentioned enzymes and other parameters such as sugar, proteins and lipids showed similar pattern in all organs studied/investigated.

When damage takes place in an organ or tissues enzymes are leaked into the blood. The increased level of some enzymes like acid phosphatase, alanine transaminase (ALT), asparate transaminase (AST) in blood is indicative of tissue damage. During the present study same pattern was observed in tissues as well as blood.

Increase in level of a particular enzyme in the blood indicates that particular enzyme is in high level in tissues. When there is toxic effect then enzymes such as, alanine transminase (ALT), asparate transaminase (AST), alkaline phosphatase, acid phosphatase leak into the blood.

During the present study when there is increase in level of acid phosphatase or alanine transminase (ALT) or asparate transaminase (AST) in any tissue then level of these enzymes is also increased in the blood. Similarly when there is diminished activity of alkaline phosphatase, the level of alkaline phosphatase is also reduced in the blood. The elevated level of acid phosphatase, alanine transaminase (ALT) and asparate transaminase (AST) in all tissues i.e. liver, kidney, brain, muscle, testes and elevated level of these enzymes in the blood are in complete accordance with each other.

When level of these enzymes was reduced in different body organs then level of these enzymes was decreased in blood also.

Increased level of enzymes such as alanine transaminase (ALT), asparate transaminase (AST) due to inhibition of membrane transport is another indication of cellular damage.

(4) In the end we may conclude that a common pattern of tissue biochemistry was observed in all organs evaluated during the present project i.e. liver, kidney, brain, muscles and testes. Level of sugar, total protein and alkaline phosphatase were parameters showed some improvement after treatment with 2 mg dose of C.
*sinensis*. All these parameters showed some improvement after treatment with 2 mg dose of *C. sinensis* but this dose was not able to prevent tissue damage caused by cadmium because all these parameters remained slightly decreased in comparison to normal control and slightly increased in comparison to cadmium control group.

Higher dose of *C. sinensis* i.e. 4 mg/100gm body weight dose was able to prevent tissue damage caused even by a higher dose of cadmium because all biochemical parameters were normal in almost all experimental animals. Similarly levels of total lipids, acid phosphatase, alanine transaminase (ALT), asparate transaminase (AST) showed increase with both doses of cadmium. Here also 2 mg dose of *C. sinensis* was not totally effective but in 4 mg dose of *C. sinensis* caused all these parameters were normal.

Same pattern of all these parameters was seen in reversibility groups where co-treatment with cadmium and *C. sinensis* were discontinued for 15 days after 15 and 30 days of cadmium and *C. sinensis* extract feeding. Here also the parameters showed slight improvement but not completely normal, in lower dose group i.e. 2 mg /100 gm aqueous extract of *C. sinensis*. The parameters were almost comparable to same parameters of non reversibility groups. Higher dose of *C. sinensis* i.e. 4 mg /100 gm body weight dose was able to prevent tissue damage indicated by biochemical parameters which were all in normal range and these levels do not show any change even after discontinuation of extract feeding for 15 days.

(5) Most probably protective effects of *C. sinensis* are by inhibiting cadmium uptake and its accumulation in various organs. Several other antioxidants such as *Coscinum fenestratum, Curcuigo orchioides, Cassia fistula, Eclipta alba, Andrographis paniculata, Ganoderma lucidum, Ganoderma formosanum, Ganoderma neo-japonicum*, glutathione, Vitamin-E, Vitamin-C, selenium and soybeans are also reported to protect tissue damage by various mechanisms.

Vitamin-E is widely recognized as an antioxidant that reduces cadmium toxicity by interfering in lipid peroxidation. This mechanism of antioxidant
activity of Vitamin-E has been observed in several systems (Chow, 1991; Sugiyama, 1994). Jia and co workers (1998a) have reported synergism of tea polyphenols and alpha tocopherol.

Ant hepatotoxic property of different species of *Ganoderma* had been attributed to their free radical scavenging properties (Jer.Min Lin et. al.1995). Hepatotoxicity preventing properties of *Coscinum fenestratum* (Venukumar and Latha, 2004) and *Eclipta alba* (Sexena et. al., 1993) have been attributed to their ability to prevent damage of microsomal membrane.

The probable mode of action is supported by the fact that when *C. sinensis* treatment was discontinued, all parameters i.e. sugar, total protein, albumin, globulin, lipid, alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase remained normal. This fact indicates that some how cadmium uptake was inhibited in the cells so toxic manifestations of cadmium were not produced.

(6) Another probable mode of protective action of green tea might be by quenching of ROS, which is mainly responsible for toxic manifestation of cadmium, as green tea is a well known antioxidant. There are evidences that reactive oxygen species (ROS) are formed in the presence of cadmium and these could be responsible for its toxic effects. (Amoroso et. al.,1982, Bagchi et. al.,1996, Thevenod and Friedmann,1999, Szuster, 2000, Clesielska et. al. 2000, Buetler et. al, 20002) It is a well known fact that antioxidants react with ROS and protect tissues against damage caused by ROS. Several protective agents including zinc, glutathione and metallothionien, play an important role in detoxification processes (Chowdhary et. al. 1987, Singhal et. al, 1987, Hayes and McLellen, 1999; Cherian, 1998; Brugnera et. al, 1994). In our body some natural antioxidants are present. There are reports that consumption of green tea results in significant rise in plasma antioxidant activity.
Another probable mechanism of protective effect of green tea might be indirect. Polyphenols and flavonols might be interacting indirectly by modifying the enzyme activity thus preventing tissue damage. Cells have developed a comprehensive array of antioxidant defensive in the form of enzymatic and non-enzymatic mechanisms, to prevent free radical formation or limit their damaging effects.

Caslino et. al. (2002) reported that cadmium causes toxicity by reducing antioxidant enzymes. Vitamin-E, besides a significant reduction in lipid peroxidases can reverse the inhibitory effect of cadmium on antioxidant enzymes (Sarkar et. al. 1998). Green tea might protect tissue damage by induction of antioxidant enzymes. Stacey and Klaassen (1988) reported that cell injury by cadmium was much reduced in the presence of manganese which is a co-factor of many antioxidant enzymes.

Catechins are known to be biologically effective antioxidants and free radical scavengers and green tea is rich in catechins such as epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechingallate (ECG), epicatechin (EC). The antioxidant activity of the green tea depends upon the amount of catechins present.