SUMMARY AND CONCLUSIONS
In the present investigation, the impact of guanidine hydrochloride (GuHCl) on some aspects of detoxification mechanisms has been studied by taking albino rat as an experimental model. GuHCl has been selected for the study because of its better solubility in water and easy entry into different tissues of the animal.

Wistar strain male albino rats (150±5g) were maintained under normal laboratory conditions and fed standard diet (Hindustan Lever Ltd., Bombay) and water ad libitum. The rats were divided into two batches of eight each and experimental rats were injected intraperitoneally with 2.2 millimoles/kg/day of GuHCl (which induced uremic symptoms, Ravishankara, 1989) for seven days. The control batch was given isovolumetric dose of saline alone for the same period. Both control and experimental rats were sacrificed by cervical dislocation three hours after the administration of the last dose of test compound. Required tissues (Liver and Kidney) were excised in cold. The tissues were weighed,
homogenized in required media and the supernatants/residues were employed for the estimation of the biochemical parameters.

The guanidine induced changes in antioxidant defense mechanisms have been studied by assaying selected detoxification enzymes. The role of glutathione metabolism in countering free radical toxicity has also been assessed.

Xanthine oxidase pathway is one of the important sites of free radical production. The superoxide anion radical ($O_2^-$) which is known to initiate the oxidation of polyunsaturated fatty acids (lipid peroxidation). Biological membranes are rich in unsaturated fatty acids. Therefore it is not surprising that membrane lipids are susceptible to peroxidative attack more so in the presence of superoxide anion radicals. Elevated xanthine oxidase activity levels found in the present study in liver and kidney of guanidine treated rat indicated increased oxyradical production in response to the daily dosing of guanidine which are responsible for oxidation of polyunsaturated fatty acids (lipid peroxidation).
Xanthine oxidase also plays an important role in disposal of ammonia converting it into less toxic uric acid. The enhanced xanthine oxidase levels in the present study under guanidine intoxication indicate effective detoxification of ammonia by this enzyme. High levels of xanthine oxidase activity observed in the tissues of albino rat suggest that tissues favour detoxification of ammonia by channeling the same towards uric acid synthesis, thereby maintaining nitrogen balance in the tissues under guanidine stress.

The free radicals are responsible for the formation of thiobarbituric acid (TBA) reactive products. TBA reaction is one of the most widely used tests for determining lipid peroxidation. Level of Malondialdehyde (MDA) in the tissues is an index of rate of lipid peroxidation. This is one of the products of lipid peroxidation which seems to be synthesized in relatively constant proportion to lipid peroxidation. In the present study elevation in malondialdehyde content was also observed in the tissues during guanidine toxicity. In consonance to this electron spin resonance spectral (ESR) analysis showed elevated free
radical generation in the tissues of guanidine hydrochloride treated rat over that of control.

Histopathological observations were made to elucidate relationship between lipid peroxidation and tissue damage under guanidine stress. The extent of liver damage observed in the present investigation is degeneration of cytoplasm, necrosis in hepatocytes, exudation of nucleus, and its degeneration and pycnotic nuclei indicate that guanidine hydrochloride treatment causes impairment to the architecture of the tissue, since liver is the major metabolic centre in the detoxification of foreign compounds. Glomerular damage, atrophied glomeruli, reduced lumen of proximal tubule, severe necrosis in the interstitial cells and detached glomerular stalk were noticed in the kidney of experimental rat. As kidney forms the main organ of excretion the changes noticed are quite prominent. During excretion the undetoxified molecules pass through kidney and appear to have caused considerable damage to tissue.

The above pathological changes in liver and kidney might be due to increased free radical production and
elevated lipid peroxidation observed in the present study during guanidine toxicity.

Glutathione, a cysteine containing tripeptide is the most abundant nonprotein thiol in mammalian cells. It plays an important role in the detoxification of xenobiotic compounds and in the antioxidation of reactive oxygen species and free radicals. The function of glutathione include maintenance of the thiols of protein and reduced forms of other compounds such as ascorbic acid and \(
\alpha
\)-tocopherol protection of cells against oxidative damage and free radical damage and other types of toxicity. Because of its multiple functions in various tissues and its involvement in many diseases and malnutrition, a clear understanding of the interrelationships among tissue glutathione and oxidative stress is clinically relevant. Glutathione levels were depleted in liver and kidney of guanidine treated rat suggesting their conjugation to the electrophilic xenobiotics and their metabolism through action of glutathione-S-transferase.

In the present study tissues showed elevated levels of glutathione-s-transferase (GST) activity under guanidine
treatment. The elevated GST activity in the tissues of guanidine treated rat indicates its active participation in the detoxification of guanidine and their excretion as corresponding mercapturic acids. GSTs catalyze the conjugation of reduced glutathione with a wide variety of electrophilic compounds and play an important role in the biotransformation and detoxification of many xenobiotics. Hence in the present study GSH levels were decreased in concurrence to the activity levels of GSTs.

Generally oxidative stress induces glutathione peroxidase (GPX) activity. In the present study activity levels of selenium-dependent glutathione peroxidase (Se-GSH-Px) increased in response to the daily dosing of guanidine hydrochloride to rats. The hepatic glutathione peroxidase was found to be more sensitive than kidney as evinced by percent change values. The elevation in Se-GSH-Px activity indicates its active participation in the decomposition of excess hydrogen peroxide and organic hydroperoxides generated via dismutation of superoxide anion by superoxide dismutase (SOD). In agreement with this the SOD activity
levels also increased in the tissues of guanidine hydrochloride treated rat.

The peroxidase activity of GST is termed as non-SeGSHPx. The activity levels of non-SeGSHPx increased significantly in the tissues of rat during guanidine treatment. The induction of non-SeGSHPx activity could be aimed at reducing the endoperoxides produced by guanidine by utilizing GSH, thereby decreasing GSH levels in the tissues of experimental rats under guanidine toxicity.

Glutathione reductase (GR) reduces oxidized glutathione (GSSG) formed via the glutathione peroxidase reaction, with concomitant oxidation of NADPH+H+. It maintains the reduced glutathione levels (GSH) in the cell. The elevation in the activities of GR suggest rapid conversion of GSH to GSSG through increased glutathione peroxidase activities leading to low GSH levels in the tissues during guanidine toxicity. GSH plays an important role in the oxidative damage and the detoxification of many xenobiotics. Hence to cope up with the reactive intermediates generated during guanidine toxicity, the GR activity levels were elevated to maintain reduced glutathione levels in the tissues of rat.
Since, xanthine oxidase is the source of superoxide anion radicals (O$_2^-$) under in vivo conditions, the increased xanthine oxidase activity in the present study indicates probable generation of superoxide anion radicals in the tissues of albino rat in response to guanidine stress. In order to find out whether the superoxide dismutase (SOD) is involved in the detoxification of superoxide anion radicals or not, the SOD activity was studied in liver and kidney of albino rat under guanidine treatment. The elevated SOD activity levels in the tissues suggest its implication in the detoxification of superoxide anion radicals generated through increased xanthine oxidase reaction in order to arrest the free radical damage to cellular organisation. In consonance to this, xanthine oxidase activity was also elevated in the present investigation and it might be responsible for superoxide anion radical generation and increased SOD activity.

Superoxide dismutase (SOD) is involved in dismutating the superoxide anion radicals to hydrogen peroxide (H$_2$O$_2$) and oxygen, while catalase decomposes hydrogen peroxide to water and oxygen. The elevated levels of SOD, observed under
guanidine toxicity may lead to increased formation of hydrogen peroxide. Hence the role of catalase in the decomposition of same has been studied in the tissues of rat treated with guanidine hydrochloride. Increased catalase activity in the present study might be due to its active involvement in decomposition of hydrogen peroxide generated during dismutation of superoxide anion radicals by SOD. Thus the high catalase activity in the tissues of albino rat under guanidine toxicity in the present investigation indicates major importance of inorganic peroxide detoxification by this enzyme to prevent the secondary effects of peroxides.

In a nutshell, the activities of hepatic and renal antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase were increased in the present study to combat free radical mediated toxicity during oxidative stress induced by guanidine treatment.

Glucose-6-phosphate dehydrogenase is an important enzyme which pioneers the HMP shunt by catalyzing reversible oxidation of glucose-6-phosphate to 6-phosphogluconolactone.
During GuHCl treatment, the activity levels of G-6-PDH were increased significantly in both the tissues studied. The common feature of the tissue under stress conditions is a general elevation in the activities of NADP dependent dehydrogenases, and reduction in the activities NAD dependent dehydrogenases. This is obvious, since one of the major function of HMP shunt is to provide reduced NADP required for the processes outside the mitochondria. Since liver is the major site of nucleic acid and fattyacid synthesis and for the detoxification of chemicals and drugs maximal increase in hepatic G-6-PDH activity under GuHCl stress may result in the production of more pentoses and reduced NADP for the synthetic and detoxification purposes, possibly to mitigate the adverse effects of GuHCl and this can be considered as biochemical adaptive phenomenon exhibited by the tissues during guanidine stress.

The levels of cytochrome P₄₅₀, a hemoprotein plays a pivotal role in the metabolism of drugs, carcinogens, endogenous and exogenous toxins, was also increased in the present study indicating induction of cytochrome P₄₅₀ dependent mixed function oxidase system during guanidine
toxicity suggesting their active participation in the metabolism of guanidine. Induction of cytochrome \( P_{450} \) could have gross effects on the organism's ability to metabolize xenobiotics since cytochrome \( P_{450} \) plays an important role in the detoxification and elimination of several chemicals. In the present study GuHCl treatment has induced the synthesis of cytochrome \( P_{450} \) levels in experimental animals which is suggestive of increased mixed function oxidase activity in relation to its increased biotransformation.

To sum up, the present findings suggest that biochemical lesions due to free radical generating systems through altered enzyme activities of glutathione metabolism are significantly implicated in the etiology of guanidine toxicity. The depletion of glutathione levels during guanidine toxicity results in increased formation of free radicals as evinced by elevated glutathione peroxidase and glutathione-S-transferase. Formation of less toxic mercapturic acid conjugates through the involvement of glutathione mediated enzymatic systems and their excretion through the urine to cope up with the continuous dosing of guanidine has also been indicated. One important finding of
the present study is the stimulation of renal and hepatic glutathione mediated antioxidant defense mechanisms during uremic conditions, suggesting a compensatory role played by thiols.