CHAPTER 7

DISCUSSION

Laboratory studies on analysis of stress responses in tissues of organisms exposed to metal can help to understand the mechanism through which metals exert their toxicity in organisms and hence the results can be used to explain the impact of heavy metal toxicity on organisms in field. To date, mercuric chloride has been studied almost extensively as model of nephrotoxicity and changes in haematological indices in a number of animal species. However, little information is currently available on the toxic effect of HgCl$_2$ on testis.

The mammalian testis provides a suitable and necessary environment for maturation of spermatozoa. The Damage induced by mercury compounds appears to be of great importance in understanding the biological effects of mercury in regard to male reproductive function.

Results of the present study suggest that exposure of rats to the two doses of HgCl$_2$ through oral administration induced male reproductive toxicity in rats. Treatment with mercury showed a decrease in testis & accessory sex organ weight. The weight reduction of seminal vesicle, epididymis and prostate may be due to mercury induced decrease in the formation or storage of their fluids. Whereas animals treated with Mercuric chloride (low dose) and Smilax china (Group V), and Smilax china as post treatment (group VIII) showed an increase in the wet weight of organs. This increase is due to a compensatory change or fluid accumulation in the testis which provides favourable environment for spermatogenesis. Also there is weight reduction in liver and kidney for the animals treated with mercuric chloride. Whereas animals treated with mercuric chloride and Smilax china showed an increase in the wet weight of these organs.
Exposure of albino rats to mercuric chloride significantly affects the spermatogenesis. Administration of heavy metals causes degenerative changes in testicular tissue and accessory reproductive organs [160]. Our study revealed inhibition of steroidogenesis and a decrease in the level of testosterone. Similarly cellular degeneration in the seminiferous tubules and Leydig cells due to administration of Mercuric chloride (0.5mg/kg) to rats which correlates with the study of Chowdhary et al., (1982) [161].

Mercuric chloride at doses of 0.5mg/kg/Bw and 1mg/kg/Bw for a period of 30 days decreases the spermatogenesis and decreases the testosterone levels, [94] which is well exhibited in the present study.

In the present investigation, when animals were treated simultaneously with mercury and Smilax chin a 400mg/kg/Bw, they showed protection against mercury induced cytotoxicity. The seminiferous tubules showed less shrinkage and reduced malformation of different spermatogenic cells. Mercuric chloride caused degenerative changes in rat testis & epididymis by lowering the level of 3beta-hydroxy-5-steroid dehydrogenase [162].

This finding is acceptable, as optimal levels of androgens are necessary for maintaining normal structure and functioning of the gonads and accessory sexual organs [163]. In this study mercury induced damage was significantly less in the presence of Smilax chin a, which reflects its androgen-like activity, as supply of exogenous androgens can restore impaired spermatogenesis by maintaining normal structure and function of gonads and accessory sexual organs [164,165].

Smilax myosotiflora can be given as aphrodisiac [166] and this is the first study reporting Smilax chin a against mercuric chloride intoxication. It also shows when the animals were treated with Smilax chin a after mercuric chloride intoxication for a period of 15 days showed marked changes in the histoarchitecture of the seminiferous tubules showing its positive affinity through the steroidogenic action.

This damage may be caused by the reactive oxygen species produced by mercury within the animal’s body. Smilax chin a interact with mercury ions, neutralize them or bind with transition metals and prevent the ROS mediated oxidative damage in testis and protect the tissue from intoxication and enhances spermatogenesis.
SOD is the first antioxidant enzyme to deal with oxy radicals by accelerating the dismutation of superoxide (O2-) to hydrogen peroxide. CAT is a peroxisomal haem protein that catalyses the removal of H2O2 formed during the reaction catalysed by SOD.

Thus SOD and CAT act as mutually supportive antioxidative enzymes, which provide protective defense against reactive oxygen species. These ROS are very unstable and highly reactive. They become stable by acquiring electron from nucleic acids, proteins, carbohydrates and lipids, there by a cascade of chain reaction are initiated resulting in cellular damage and causes lipid peroxidation [167].

Thus in the present study chronic administration of HgCl2 causes decrease in the levels of SOD and Catalase. Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids (PUFAs) and its occurrence in biological membranes causes impaired membrane function, structural integrity, decrease in membrane fluidity and inactivation of a several membrane bound enzymes. [168].

Thus, it is plausible to speculate that mercury treatment may result in peroxidation of PUFAs, leading to the degradation of phospholipids and ultimately result in cellular deterioration in the testis. Various studies also suggested that a strong correlation exists between mercury induced toxicity and the induction of LPO [169]. The present data revealed significant increase in lipid peroxidation level which is due to oxidative stress resulting in cellular damage and in Co-administration of *Smilax china* with low dose of HgCl2 exposed groups exerted amelioration effects.

This antioxidant and ROS scavenging effects of *Smilax china* is only due to its phenolic (-OH) group, which would inhibit the -SH group oxidation and block thiol depletion and thus it protects the oxidation of protein.

Further it also enhances the activities of some antioxidant enzymes such as SOD and catalase is in agreement with the previous findings [167]. Chronic exposure to stressful situations induces a highly complex set of neuroendocrine changes, dependent on the interaction between individual characteristics and situational factors [170].
During stress, the hypothalamic-pituitary–adrenal axis is activated, the glucocorticoid secretion increases [171], and consequently circulating testosterone levels are decreased via glucocorticoid receptors in leydig cells [172]. Low testosterone production adversely affects the quality of semen and subsequent fertility of males. Animals treated with mercuric chloride show increased levels of cortisol as stress response, where as animals treated with *Smilax china* showed marked decrease in values of cortisol levels (*p*<0.01).

The administration of mercuric chloride causes decrease in circulating testosterone level in dose of 0.5mg/kg/Bw and 1 mg/kg/Bw for 30days which correlates with the earlier study of Ramalingam et.al.,2003 [172] .The animals receiving *Smilax china* as prophylactic dose considerably maintains the testosterone level and animal group receiving *Smilax china* as post treatment also showed significant level of the hormone (*p*<0.001). In low dose affected group post treatment of smilax china was highly significant (*p*<0.001).

The changes in the hormone levels caused by the mercuric chloride suggest the dysfunction of pituitary-testicular axis, where the prophylactic and therapeutic effect of *Smilax china* on low dose affected mercuric chloride group is due the steroidal compounds which are present in it. The reduction in the diameter of seminiferous tubules of the rat testis is because of severe atrophy of the tubules, low spermatogenic epithelium, and histological disruption of spermatogenesis induced by mercuric chloride.

This is in concordance with the findings of Hyung et al. 2004., [173] who observed a reduction in the diameter of the seminiferous tubules after neonatal exposure to Dibutyl phthalate (DBP), which is due to loss of the germ cells from the seminiferous tubules of the testis.

Ki-67 is a nuclear protein that is associated with and may be necessary for cellular proliferation. It is strictly associated with cell proliferation. During interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). Animals treated with mercuric chloride showed markedly decreased expression of
Spermatogonia. Where as animals treated with mercuric chloride and *Smilax china* showed mild decreased in the expression of ki67. Animals on post treatment with *Smilax china* (Group VIII) showed normal expression of ki67 as that of control showing the positive affinity of the drug towards mercuric chloride intoxication.

Directly or indirectly, the Oxidative stress plays a major role in pathophysiology of reproductive dysfunction [174]. In this study, there is decline in the sperm count in animals treated with mercuric chloride. This is due increased Oxidative stress or defects in the protective function of the epididymis [175]; this could have caused sperm cell DNA damage or apoptosis.

Sperm counts were significantly increased in animals treated with Smilax china; this might be as a result of reduced sperm cell apoptosis in testis and improved epididymal sperm maturation through increased antioxidant system [176].

Sperm are more vulnerable to excess ROS because of high amount of polyunsaturated fatty acid present in their plasma membrane [177]. Increased LPO and altered membrane function can render defects in head (amorphous, microcephalic and acephalic), neck and tail of sperms.

Animals treated with mercuric chloride showed decrease in actively motile sperms and increase in the percentage of sluggishly motile sperms and non-motile sperms. Whereas animal treated with mercuric chloride and *Smilax china* (Group IV and V) and also in *Smilax china* as post treatment for animals affected with mercuric chloride toxicity (Group VI and VII) there is marked increase in the total count of the sperm with increase in the percentage of the active motile sperm and decrease in the percentage of sluggishly motile sperms and non-motile sperms showing its steriodogenic activity.

There was also significant effect of the mercuric chloride on the sperm morphology abnormalities. Abnormalities such as pin heads, double heads and round heads were detected on the sperm cells of the animals treated with mercuric chloride. Animals when treated with *Smilax china* along with mercuric chloride (Group IV and V) and as post treatment (Group VI & VIII) exhibit markedly decrease in abnormalities of the sperm morphology.
Results of the present study indicated that mercuric chloride affected the histological structure of prostate in albino rats. The prostate showed degeneration of epithelial cells, cytoplasmic vacuolization, moderate fibrosis, flattened epithelium and congestion of blood vessels. Whereas animals treated with *Smilax china* along with mercuric chloride (Group IV & V), also as post treatment (Group VI & VIII), showed minimal congestion, minimal fibrosis, hyperplastic epithelium showing the positive sign of new cell formation. This is the first study reporting the protective effect of *Smilax china* against mercuric chloride intoxication in secondary sexual organs.

Also animals treated with mercuric chloride showed changes in epididymis showing dilated lumen, marked cytoplasmic vacuolation in the epithelium and lumen showing decreased amount of sperm, showing the result correlating with the sperm analysis, since there is decrease in the sperm production in the seminiferous epithelium. Animals with *Smilax china* as post treatment showed minimal cytoplasmic vacuolation in epithelium and mild decrease in the sperm with in the lumen, showing the protective effect of *Smilax china* on mercuric chloride intoxication.

Seminal vesicle of albino rats treated with mercuric chloride showed marked Histopathological changes. Vesicles are dilated, cytoplasmic vacuolation in epithelium and fibrosis are seen. But animals receiving *Smilax china* along with mercuric chloride (Group IV & V), also on *Smilax china* as post treatment both on High dose affected and low dose affected group (Group VI & VIII), the dilatation is minimal, desquamation of epithelial cells are seen denoting the shedding of the older cells and formation of the new cells, denoting the protective effect of *Smilax china* on seminal vesicle.

Administration of mercuric chloride elevated the serum levels of SGOT & SGPT, significantly due to its enzymatic activation of CCl•3 free radical, which in turn alters the structure and function of liver cells [178]. The results of the present study reveal that methanolic extract of *Smilax China* roots (400mg/kg/Bw) exhibited protective action against mercuric chloride induced liver damage in a dose related fashion.

The amelioration of liver toxicity by the test extract was evident from its significant effect on serum enzyme levels and morphological parameters. These findings were further supported by histopathological observations. Further, preliminary photochemical
investigation revealed that the extract showed presence of flavonoids, tannins, alkaloids, saponins and glycosides.

The literature has already documented the antihepatotoxic value of flavonoids (Singh B et.al., 1988) [179]. Thus, it appears that the hepatoprotection offered by *Smilax China roots* extract is due to its flavonoid content. The study of Stachiotti et.al., 2004 [180] revealed that HgCl$_2$ causes periportal fatty degeneration and cell necrosis in the liver, which is also evident in our study. Necrotic lesions may be due to progressive degenerative action of intracellular enzymes of the injured cells [181]. Animals treated with *Smilax china* showed decreased intracellular damage.

Mercuric chloride showed dose dependent toxicity. The administration of mercuric chloride shows significant decrease in the values of haemoglobin, RBC and PCV. Heavy metals disturbed the formation of hemoglobin due to which their percentage level is decreased in the blood. Similar results were observed by Mathur et.al.,(2002), [182] and Christensen., et.al., (1977) has reported that there was significant reduction in hemoglobin percentage after exposure to heavy metal [183].

The reduction in the hemoglobin percentage is due to production of reactive oxygen species under the influence of mercuric chloride, which results in destruction of the red blood cell membrane and its function. Whereas *Smilax china* treatment in experiment, increases the level of hemoglobin to certain extent because of its natural antioxidant function.

Enhancement in the total leucocyte count following mercuric chloride intoxication is due to leukocytosis, as leucocytosis is an outcome of proliferation of haemopoietic cells leading to progressive infiltration in peripheral blood [184,185] which correlates with our study. However *Smilax china* tends to prevent the intoxication on low dose affected (p<0.01) mercuric chloride group, when compared to the high dose affected group.

Exposure to different concentrations of mercuric chloride causes renal damage. Inorganic mercury has been shown to accumulate in the renal cortex and affect the morphology and function of the tubules[186]. The degree of necrosis in proximal tubules were higher in High dose administered Mercuric chloride group, whereas animals treated
with *Smilax china* showed decreased histopathological changes which was correlated with the biochemical values.