Chapter IV

Discussion
DISCUSSION

4.1-BEHAVIOR DURING EXPERIMENTATION:

4.1.1- PYRETHRIN:

The results depict the effects of various doses of pyrethrin on the behaviour particularly on locomotion, aggressiveness and their attitude towards their eating, drinking habits etc. Initially there is an increase in the locomotory activities like jumping, running and continuous hiccups. Increase in doses (with days) of pyrethrin aggravates these movements. But after the interval of 40 days locomotary activities become slow leading to delayed response to food and water. Sometimes they rub their front legs so vigorously that even bleeding occurs. Pyrethrins are well-established neurotoxins. The literature points to fact, that these compounds increase cholinergic and dopamine receptor in the cerebral cortex and increase the activity of the receptors. There-by causing changes in their behaviour (Eriksson and Nordberg, 1990; Hussain et al., 1996; Lazarini et al., 2001). Hussain et al. (1996) pointed out not only increased level of catecholamines, but also sensitivity of the receptors under the influence of pyrethroid. Saxena and Sharma (2000); Hoy et al. (2000) also described almost similar changes in the behaviour of albino rats with low doses of
fenvalerate given up to 21 days. They speculated that the metabolites formed due to activity of cyno group could be responsible for such changes.

Another explanation given by Tabor and Tabor (1983); Schuber (1989); Iqbal and Koeing (1985) that pesticide specially pyrethroid are associated with synaptic transmission and modulate the binding characteristic of several different neurotransmitter receptor-ionophore complexes in rat brain. This view was further strengthened by the findings of Gilad et al., 1992 and Righi and Palermo-Neto, 2003. The cyno group of pyrethrins plays a key role in induction of adrenal medulla and the basal ganglion of the brain to release acetylcholine and dopamine respectively (Boer et al., 1988 and Keichiro et al., 1990). Neurotransmission is also affected by disturbance in Na⁺ gating. The increase in Na⁺ channel plays an important role in the release of catecholamines and adrenalin from nerve terminals (Eells et al., 1992). The prolong effect proved to be deleterious because of the accumulation of unmetabolised pyrethrins in the CNS and more especially in cerebellum, hypothalamus and cerebrum (Ray, 1982).

Taking into account the toxicity of the pyrethroid on liver, adrenal gland, and thyroid gland etc. the change in their appetite, locomotion and abnormal movements can be correlated. Loss of appetite, loss of weight can be corroborated with liver damage, while the impaired thyroid function may be responsible for inarticulate movements. The abnormal behaviour is concerned with the activity of adrenal gland, through muscaranic and callanergic modulation (Moniz et al., 1994).
Eriksson and Nordberg (1990) found an increase in the muscarinic acetylcholine receptors in the cerebral cortex of neonatal mouse brain following deltamethrin and bioallethrin (a Type I pyrethroid) exposure. Further, in 4 months old mice, a tendency towards a decline in the amount of H-quinuclidinyl benzilate-binding sites in the cerebral cortex was reported by Eriksson and Fredricksson (1991) following deltamethrin (0.7 mg/kg bw) exposure, between postnatal days 10 and 16. This is generally considered that their particular sites do play a definite role in fixing up the behaviour of the animal. If their receptors are altered in post-natal day, deviation in behaviour during adulthood is eminent. These differences may be explained by species or dose difference (mice versus rat or 0.7 versus 7mg/kg bw). In the present study, exposure to deltamethrin was given only after weaning, when the neuronal plasticity and homeostatic ability of the brain had altered and henceforth adverse effects on certain behaviour were monitored i.e. locomotion and learning (Kolaczinski and Curtis, 2004). Further, it is well established that Type II pyrethroids prolong the gating kinetics of sodium channels thereby disturbing the whole cascade of neurotransmission process i.e. membrane depolarization, firing rates of neurons, release and uptake of neurotransmitters in the synaptic cleft and changes in Ca^{2+} efflux (Eells and Dubocovich, 1988; Eells et al., 1992; Shafer and Meyer, 2004).

The interaction of deltamethrin with cholinergic and dopaminergic receptors may be a consequence of its primary action on regional brain polyamines. Changes in the physiological concentrations of these
polycations in various brain regions as observed in the present study may adversely affect Ca\[^{2+}\] homeostasis, experience dependent brain growth, neurotransmitter uptake etc., which cause a rearrangement in overall synaptic events (Shaw and Pateman, 1973; Iqbal and Koenig, 1985; Ferchmin and Eterovie, 1987). Iyaniwura and Okonkwo (2004) observed that the behavioural changes in cypermethrin-exposed animals may also be due to altered cholinergic and dopaminergic receptor responses in weaning rats.

4.1.2- FERNOXONE:

Fernoxone slowly ceased most of the animal’s spontaneous activity. Animals sit still and when they are induced, they made a sudden movement such as a quick start or a righting motion. There limb spread out and they fall. Hind limbs are the more affected then the fore limbs. If incited to continue their attempts to move they finally recover. Initially the motion is slow and upward. But continue efforts lead to normal speed with smoothness.

WHO (1984) published that on acute administration of 2,4-D results in mytonia syndrome. However, in this study there is no clear-cut chronic syndrome has been produced by continued administration of LD\(_{50}\) of 2,4-D per day for 3 months. Oliveira and Palermo Neto (1995) suggested that
after injecting 2,4-D body weight of rats was reduced and there was less displacement and more self-directed behaviour. Borotolozzi et al. (1998) observed that adult rats of both sexes showed excessive grooming and rearing.

This can be presumed that 2,4-D affects the normal neurology furcating, neuromotor reflexes and spontaneous motor activity. This can be correlated with Ca, Na and K ions gating in cerebral neurons or hypersecretion of cerpodopamine and hyperactivity of cholenergic receptors etc.

Bucher (1946) observed many behavioural changes with 2,4-D and correlated these activities with actual intoxication causing to damage liver, kidney and spleen. Lakshmana and Raju (1996) in their classic study noted impairment in learning ability and loss of weight, which they explained/correlated with high amount of monomines and low activity of cholinesterase. They also assessed the behavioural changes with over secretion of norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT) and supported their finding with histopathological changes like vacuolation and fissure formation in brain stem and hippocampus lobe of cerebrum.
4.2-EFFECT OF PYRETHRIN ON ENZYMES:

4.2.1-Serum Glutamate Oxalate Transaminase (SGOT/AST) and Serum Glutamate Pyruvate Transaminase (SGPT/ALT):

From the table 1 and 2 it becomes clear that the pyrethrin has deleterious effect on liver and causes to damage to hepatocytes. Hence releasing the higher concentration amount of liver enzymes in blood. The hepatocytes are enzymatically highly active cells and rich in GOT and GPT to meet metabolic stresses. Upto 5th day pyrethrin, though it affects liver yet the values of SGOT dose not increase significantly. Possibly, the initial doses are counteracted by body’s defense system or its binding with serum albumin hence the damage is not much to affect the amount of enzyme significantly. From 10th days of pyrethrin treatment, the enhanced level of enzyme in blood shows its detrimental effect on hepatocytes. This may be because of pyrethrin itself or by its some metabolite. Histopathological studies of liver (photographs are not given here) point to the fact that this toxicant first affects the membrane of the cells resulting in the leaching of the enzymes in blood. Level of SGPT though increases after the 1st day treatment but the significant changes appear only after 20th day of the treatment. The amount of the enzyme increases with the days of pyrethrin treatment. The highest average value 71.79u/l was recorded on 150th day, which were almost 3 folds more than the normal values.
The results of present study are supported by the findings of many researchers. Ahmed (1986); Gupta (1988); Ahmed et al. (1989) reported that cypermethrin increases the level of SGOT and SGPT. They observed histopathological changes in vital organs of rats. After cypermethrin, intoxication liver tissue injury is the cause to rise the SGOT and SGPT level in blood (Ahmed and Gupta, 1988).

El-Tawil and Abdel-Rahman (2001) suggest that the cypermethrin was cytotoxic to rat hepatocytes even at the concentrations of 200mg/ml. Toxicity was measured by a decrease in cell viability and leakage of ALT and AST enzymes into the culture medium by them. Singh and Sharma (2000) show a significant augmentation in the level of SGOT and SGPT due to the hepatotoxic effect of alphamethrin in cockerels. Similarly, Shukla (1991) shows in his toxicological study with deltamethrin in buffalo calves that there is an increased in the serum transaminases (ALT and AST) due to the cellular injury to hepatic parenchyma and other tissues. Permethrin and benenil also increase ALT and AST level (Shah and Gupta, 1997; Homeida et al., 1981). Increase in serum ALT and AST is the toxic effect of diminagene observed by Nanda et al. (1996). Majumder et al. (1997) describe the effect of short-term dermal toxicity of fenvalerate and residue on cell architecture and biochemical profiles in broiler chick. They observe that fenvalerate increases ALT and AST activities in all the tissues except brain. The findings in this study are also in accordance with those of
Reddy et al. (1989) who noticed a progressive increase in the activities of SGOT and SGPT and corroborated this rise with liver damage because of the compound. Thus, alterations in liver function occur because of pesticide treatment and the enhanced level of the enzymes reflects liver damage (Balazs, 1981).

4.2.2- Alkaline phosphatase:

Liver is the site for detoxification, to combat and eliminate the bacteria and antigens, which come from alimentary canal. Every day many cells die in natural course and during fighting as a defensive mechanism of the body. This is the reason so that alkaline phosphatase concentration remains in a definite range blood. The substances like pyrethrin, fenoxone and many others compounds cause much more damage to liver and other tissues of different organs. Thus an elevated level of alkaline phosphate is another indication of liver toxicity. In healthy albino rats, its concentration ranges from 1.25u/l to 2.96u/l, on pyrethrin exposure the enzyme level increases from 5th day, it is 4.74u/l. The concentration of the protein in consideration gradually increases and on 150th day after treatment, it becomes just double, than the amount in control healthy rats. From 10th day upto 120th day a gradual increase in alkaline phosphatase is observed. This remains in an almost close range. Which can be explained on the basis that the enzyme is being utilized to produce much energy to neutralize the effects of the toxic substance, and in regeneration of the tissues. As stated
earlier a changed level of the enzyme is because of injury to the hepatocytes or increased permeability membrane under the influence of pyrethrin. Since alkaline phosphatase is a membrane bound enzymes, any change in membrane permeability may result in the leakage of this enzyme into blood leading to an increase in its level in the blood.

Many researchers have noticed such increases in serum alkaline phosphatase due to permeability change. Gupta et al. (1991); Parker et al. (1984) and Hundal et al. (1998) supplemented the finding by reporting increase in hepatic alkaline phosphatase activity in rats exposed to fenvalerate and explained the rise in alkaline phosphatase on the basic of toxic effect of the compound on liver to cause injury. The finding gets further support from Singh and Sexana (2001) who also described a rise in alkaline phosphatase after cybil intoxication in rats. According Kaur and Sandhu (2001) to sub acute oral toxicity of cypermethrin and deltamethrin elevates plasma level of alkaline phosphatase in buffalo calves.

On the contrary, Rani and Dua (1999); Das and Mukherjee (2003); el-Demerdash et al. (2003) show that the level of alkaline phosphatase is reduced when liver is exposed to the active toxicity of cypermethrin and also exhibit an increased level of lipid. Their studies did not include histopathological observation. My studies are conveniently acclaimed both by histologically and by measuring the enzyme level that liver is affected negatively and cytolysis is more than cytogenesis.
4.2.3- Lactate Dehydrogenase (LDH):

Treatment of pyrethrin increases LDH in the blood serum of the experimental animal. It was observed that there was a decrease in oxygen consumption with an increase in concentration of pesticide. The changes in the level of blood LDH on one hand may be associated with muscles activity, myotonia, and impaired glucose metabolism leading to more synthesis of lactic acid in muscles and to the other hand can be interpreted with hepatocytosolic release of LDH.

The minimum range of LDH in the healthy untreated rats is found to be between the value 50.0u/l to 63.8u/l, while in pyrethrin treated albino rats, it significantly increases from 10th day. The highest value 106.25u/l is recorded on 150th day exposure of pyrethrin. Correlating these values with change in muscle activity (mention earlier) it becomes clear that the increase in LDH is because of liver injury. Yet, impaired glucose metabolism in muscles due to pyrethrin cannot be ignored. Probably because of some factors muscles gets much more activated thereby more lactic acid formation takes place and accumulates in muscles. Unfortunately, not much work is available on LDH in reference to rats so far.

Cremer and Seville (1982) compared the toxic effect of two pyrethroids, deltamethrin and cismethrin in rats. They observed high level of LDH in the both cases. Sonni and Galbo (1980) correlated increasing
level of LDH in cismethrin treated rats, after the pyrethroid treatment. Astonishingly, they also reported low level of LDH but after cismethrin injection the rats invoked to run fastly. They could not find satisfactory reason for the change in the LDH concentration in two conditions. Tian (1993); Radhiah and Rao (1990) measured the level of LDH and found its significant increase in the lungs and also a change in glycogen metabolism of muscles of the experimental animal in respect to the toxicity of fenvalerate. Das and Mukherjee (2003) observed the effect of cypermethrin on LDH activity. In brain and liver it was elevated, but low in kidney.

4.2.4- Cholesterol:

Peroxisomes in liver proliferate upon receiving high fatty acid diet. Their function is to breakdown the fatty acids. The proliferations of hepatic peroxisomes have been reported by also certain pesticides. It is also a major site of cholesterol synthesis through hydroxy methyl glutamate CoA pathway. The inhibitions or activation of the enzyme system is responsible for the change in the amount of the free cholesterol in blood. Cholesterol is required for steroid, bile duct, and plasma membrane formation. It is known that many pesticides cause to elavate cholesterol concentration in blood. The reason is still to be explored. In this study, the pyrethrin causes to increase the serum cholesterol level from the day 5th, it remain high up to 20th day. An initial and gradual increase in the amount of the metabolite is
observed up to 20\textsuperscript{th} day of the intoxication. After this it declines and comes to normal serum level in 150\textsuperscript{th} day.

Shah and Gupta (1997) observed that permethrin produces marginal increase in serum cholesterol level at lower doses whereas at higher doses it significantly reduces cholesterol concentration. Majumder et al. (1997) reported that fenvalerate decreases the level of cholesterol in the kidney and also in brain tissues after the treatment of 0.1\% concentration of fenvalerate. Contrary to it Mandal et al. (1996) emphasized that no change in serum cholesterol amount takes place in black Bengal goat after fenvalerate administration. However, the data of cholesterol with reference to liver damage can be possibly concluded with reference to peroxisomes.

The reduction in serum cholesterol level could possibly be due to either a decrease in the synthesis and/or increase in the breakdown of fatty acids due to proliferation of peroxisomes. Many hypolipidemic/hypocholesteremic drugs and certain pesticides have been reported to induce hepatic peroxisomal proliferation (Cohen and Grasso, 1981; Reddy and Lalwani, 1983; Kawashima et al., 1984; Yousef et al., 2003). The peroxisomal proliferation possesses hypolipidemic property and plays a role in lipid metabolism. Beta-oxidation of fatty acid may lead to induce hepatocellular carcinomas in rats (Lalwani et al., 1981; Reddy and Lalwani, 1983; Locke et al., 1989).
4.2.5- Glucose:

Sacktor et al. (1966); Nahorski et al. (1970); Ray and Cremer (1979) observed mark hyperglycemic condition with pyrethrin treatment. The result given here are in confirmity with their findings. The normal range of glucose concentration in healthy rats is between 41mg/dl to 68mg/dl. While, in pyrethrin treated rats it starts increasing right from day one (74.25mg/dl) and found to be elevated up to the last day (113.20mg/dl) of experimentation. The concentration was 3-4 times more than the normal amount in untreated rats. The increase in glucose level is to meet glucose demand of nervous tissues, which, is affected first, and exhibited unnatural gating of ions from neurons there by disturbing normal nerve conduction (Wallwork and Malone, 1971). This results in producing the convulsions and also glucose demand of the tissue.

Biochemical changes in rat cerebrum following cypermethrin administration require high amount of energy, which, can be afforded by glucose oxidation only (Edward and Berry, 1981). Cremer et al., (1980); Cremer and Seville (1982) though recorded modest rise in blood glucose but emphatically correlated with spike discharges and regionally selective glucose metabolism in brain.

The findings with LDH can be related here. The increased synthesis of lactic acid due to impaired metabolism of glucose in muscles. The high amount of lactic acid is converted back to glucose in liver and releasing it in blood to elevate its level.
Fenvalerate, another synthetic pyrethroid, is reported to cause reduction in the hepatic glycogen concentration in chicks (Majumdar et al., 1994). Hyperactivity of adrenal gland induced by permethrin or by its metabolites cannot be ignored. Adrenalin secretion is also responsible for rise of sugar in peripheral blood vessels (Weinstock et al., 1980; Goyal et al., 1986). Hyperglycemia is reported in calves following exposure to deltamethrin (Shukla, 1991). The glycogenolysis may be one of the factors for hyperglycemic condition. It is therefore, presumed that the hyperglycemia induced by permethrin in rats was possibly due to increased adrenal gland’s activity through glucocorticoids.

β cells in pancreas show progressive degeneration with dose and day’s. Obviously, it will cause the deficiency of insulin, which may be responsible for rising glucose level. As already mentioned the liver gets damaged, resulting in disturbed general metabolism viz. glycogenolysis, neoglucogenesis and lipogenesis. Thus the hyperglycemic condition with pyrethrin cannot be attributed to one condition only but ought be considered as the synergistic effect of more than one phenomenon. Bradbury and Coats (1989); Krishnappa et al. (2000) also reported high glucose serum level with pyrethroids, but no conclusive suggestion has been given by them for hyperglycemia. One of the probable causes of rat’s mortality can be presumed with hyperglycemic coma.
4.3-EFFECT OF FERNOXONE ON ENZYMES:

4.3.1-Serum Glutamate Oxalate Transaminase (SGOT/AST) and Serum Glutamate Pyruvate Transaminase (SGPT/ALT):

From the results it is concluded that the level of SGOT was significantly increased as compared to SGPT by the injection of fernoxone intraperitonealy. As fernoxone affects the hepatocytes membrane therefore, the liver enzymes pour into the blood and raise the level of SGOT and SGPT.

Berwick (1970) found that general damage of liver should be indicated by marked elevation of SGOT and SGPT. Greig et al. (1973) and Zinkl et al. (1973) explain that there is an increase in the level of SGOT and SGPT and further indicates that there is a decrease in liver functions. Abnormal, SGOT and SGPT levels due to acute poisoning with occupational overexposure to 2,4-D were reported by Bashirov and Ter-Bagdarasova (1970), Berwick (1970), Lukoshkina et al. (1970), Brandt (1971) and Prescott et al. (1979).

The oral administration of 625 mg of 2,4-D to rats is responsible for the elevated level of SGOT but in the case of SGPT it decrease (Szocs et al., 1970). Chaturbedi (1993) with herbicides observed increasing trend in the level of SGOT and SGPT, which was dose dependent. Miranda et al.
(1997) show that acute exposure of 2,4-D increases AST and ALT and sub chronic exposure increases AST activity. It was observed that 2,4-D does not accumulate in the body as revealed by chromatographic analysis.

4.3.2- Alkaline phosphatase:

By the treatment of 2,4-D or fernoxone in albino rats, the level of alkaline phosphatase increases. Alkaline phosphatase is a membrane bound enzyme. Fernoxone affects cell membrane permeability of hepatocytes that leads to leakage of this enzyme into the blood hence its level increases.

Charles et al. (1996) reported the increased level of alkaline phosphatase after the treatment of 2,4-D in rats. The biochemical analysis of blood serum constituents reveals that dioxin increases the level of alkaline phosphatase (Tryphonas et al., 1984; Rier et al., 2001). Paulino and Palremo-Neto (1995) further support these results after acute toxicity of 2,4-D (100-600 mg/kg) in cattels by monitoring the levels of alkaline phosphatase and various liver enzymes.

Paulino and Palremo-Neto (1991) investigated the acute effect of 2,4-D on the activities of alkaline phosphatase in male Wister rats, which were given a single oral dose of 0.6 g/kg of body weight (close to dose limit of LD₃₀). They found that one to four folds increase in the serum limit of alkaline phosphatase in 5, 8 and 24 hrs. treatment.
4.3.3- Lactate Dehydrogenase (LDH):

The treatment of fernoxone shoots up the level of LDH in the albino rats. 2,4-D is cytotoxic to the hepatocytes, as indicated by leakage of LDH thereby abruptly raising its amount in blood. Its effects were dose and time-dependent. Cell death was accompanied by depletion of intracellular glutathione and increase in oxidized glutathione (Palmeira et al., 1994). Wells et al. (1981) and Freisen et al. (1990) reported the increased LDH activity in severe poisoning of 2,4-D in rats.

However, it is difficult to get convinced with Abdel-Nabi (1998) who showed the decreased activities of LDH in the serum of herbicides treated adult and treated senile rats.

4.3.4- Cholesterol:

Fernoxone treatment causes to increase cholesterol level in blood of rat upto 5th day, which again increases from 120 days. In this span of 5th and 120th days cholesterol reduces but still higher than the normal concentration. It is difficult to suggest as to why cholesterol level decreases again and increase thereafter. Possibly glutathione, superoxide dismutase, and hypersecretion of steroid antagonize the effect of pyrethrin for sometime. Once such protective enzyme system, which inhibits hyperoxidative phosphorylation, are lowered due to the influence of fernoxone, cholesterol level again adopt increasing course.
2,4-D is known for their toxicity on various organs including liver and even for oncogenicity. From the total mean we concluded that cholesterol level increases when the site of synthesis of the sterol is damaged. Serum cholesterol level is increased in the rats treated with fenoxone. Albro et al. (1978), Schiller et al. (1985), al-Turk et al. (1988), Lakshman et al. (1989), and Hassoun et al. (1996) observed elevated circulating cholesterol concentration in TCDD-treated rats. Increase in the serum cholesterol was also recorded by Moore et al. (1979).

Golden Syrian hamsters exposed to 1000 µg TCDD / kg body weight, orally or intraparitonelly, had elevated cholesterol level upto 20\textsuperscript{th} days after exposure but acheived normal level on 50\textsuperscript{th} day. (Olson et al., 1980-a and Olsen et al., 1980-b)

\textbf{4.3.5-Glucose:}

Glucose level in treated rats does not show any definite pattern of rise and fall. On the contrary, it shows an aberrant level in blood. A significant rise is observed from day 1\textsuperscript{st}, which may be perhaps, due to hypersecretion of catecholamines or rise in glucocorticoids level. The increase in glucose is to meet the stress of the body and the over excitation of the neurons because of the influence on metabolism of fenoxone or other chlorophenoxy herbicides (Enan et al., 1992).
From 10\textsuperscript{th} day, the level gradually declines and comes to almost normal after chronic treatment of 150\textsuperscript{th} days. If these results are studied with, reference to histological studies of pancreas in which $\beta$ cells do not show any sign of emulation or hyperplasia. Than, it seems that the herbicide possibly is responsible to secrete more glucogenic hormone from the pituitary or it is acting upon the receptors of the hormone on $\beta$ cells. It can also be presumed that Glut-receptors in liver, muscles and kidney become hyperactive to absorb more glucose causing depletion of serum glucose level. Olsen et al. (1994) studied the regulation of glucose with 2,4-D derivates. They observed high level of insulin, corticoids and bulyezenthene. Bulyezenthene transports glucose to adipocytes. According to them Glut 4 and Glut 1 become hyperactive. $T_4$ level if declines, the ability of cell for glucose absorption is also affected, but in our histopathological study $T_3$, $T_4$ level cannot be presumed to rise as fernoxone dose not affect the amount of colloid in thyroid follicle and it is normal in the thyroid follicles' lumen.

4.4- HISTOLOGICAL EFFECTS AFTER PYRETHRIN AND FERNOXONE TREATMENT:

4.4.1-Brain: (Fig- 3.1 to 3.12)

Toukhy and Girgis (1993) described inhibitory effect of cypermethrin on the activity of the total ATPase in rat liver. This causes to disturb the transport of Na\textsuperscript{+}, K\textsuperscript{+} and Mg\textsuperscript{++}, which results in the pathological
changes and even leading to the atrophy of the cell. Rao and Rao (1995); Imamura et al. (2002) found increased activity of acetylcholine esterase in cerebral cortex, cerebral striatum, hypothalamus and in the area of hippocampus on cypermethrin administration. In this study, changes in Purkinje cells, in the cerebellum were observed. Cytoplasm of scattered pyramidal cells of CA3 hippocampus layer was vacuolated and showing focal picnosis. The neurocytes of nuclides lattices, hypothalamic and cerebral cortex had picnosis as well as complete destruction in other neurocytes at some places.

But Anadon et al. (1996) observed multiple changes in the various part of the brain with deltamethrin besides disturbance in Na\(^+\), K\(^+\) and ATPase activity. They also found inhibitory effect on Ca\(^{++}\) bound ATPase. Ca\(^{++}\) had plain important role in nerve conduction and other activities. Inhibition of ATPase causes disruption in normal metabolism of neurocytes. Greater the disruption leads to affect other metabolism and it would be proved deleterious to the cells. Their finding suggests conservable diffusion of the pyrethroids in the nervous tissue and also rapid absorption in cells. Considerable concentration of deltamethrin in hypothalamus, cerebellum, frontal cortex, hippocampus, medulla oblongata and caudate putamen was estimated by them, interestingly the retention time of the compound in these nervous part was quite longer as compared to other tissues. It also shows the high affinity of the pyrethrins to nervous tissue.
Administration of deltamethrin (5 mg/kg) for 21 days produced region specific changes by the dealkylation of ethoxyresorufin and pentoxyresorufin in rat brain with significant induction occurring in the activity of cerebellum, hippocampus, medulla-pons, hippocampus, hypothalamus, corpus striatum and mid brain (Dayal et al., 2001; Eriksson and Nerdborg, 1990).

The behavioral changes such as rhything motion, body tremors and choreoathreosis are because of this chemical affinity. It could be presumed that the some enzyme system of neurocytes is acting upon the pyrethroid and converts it to some unwanted metabolite. The cumulative effect of the given compound and its metabolite is that the cells system of hypothalamus and hippocampus are much more affected. These results are in conformity with the findings of Anadon (1991). Fenvalerate hinders, growth rate, and brain development in postnatal rats. In adult, it reduces the thickness of cerebral cortex and causes the irregular deposition of Purkinje cells. Pittman et al. (2003) provided further evidence and showed that permethrin can cause Parkinson's disease by producing primary neurodegenerative substrate.

Cypermethrin are much small molecules, which are allowed to pass through blood-brain barrier even at small concentration and the compound after crossing the endothelium having access to brain tissue. Entry to neurocytes is perhaps, facilitated through phospholipids and
sphingomyelins, which constitute the major part of white matter and present in neurocytoplasm also. Pyrethrin has greater permeability across the endothelium and zonula occludens, which finally cause the destruction of neuroglia including neurocytes. It can be very well correlated with the finding that the compound has high affinity with the neural tissue. Hence it comparatively exhibits very high metabolic disorders and similar negative effects. The results of Wu and Liu (2000) indicate that deltamethrin induces degeneration and apoptotic cell death in rat brain, suggesting an important role played through apoptosis in neurotoxicity of deltamethrin.

The findings of White et al. (1976); Gupta et al. (1999); Abou-Donia et al. (2001); Sinha et al. (2004) support this contention. They observed blood-brain barrier permeability of cypermethrin they also reported that it requires longer period of excretion from nervous tissue. The oxidative damage in the brain may also be caused by decreased level of brain GSH, an important enzyme to prevent the binding of free oxygen with fatty acids and other metabolites.

According of Kaul et al. (1996), the unco-ordinated tremors in rat occur when poisoned with pyrethroids and DDT, separately. Narahashi and Heas (1968) and Narahashi et al. (1971) reported that the effects on the nervous system of the two poisons are similar. It is interesting to note that however much higher concentrations of DDT occurs in the brain of rats at the time of death [1.4 μmol/g (14)] than in those which dosed with
cismethrin (0.013 μmol/g). Now it can be opined that pyrethroids and pyrethrin affect nervous system in multiple ways viz. disturbing Na⁺, K⁺, Ca⁺⁺ and Mg⁺⁺ gating, inhibition of glutathione, cyclic GMP, unusual oxidative, phosphorylation, inhibition of 3-HDL glutamate uptake (Zhao et al., 1995) and toxic metabolic pyrethrin itself changing synaptic nerve transmission of impulse (Aldridge et al., 1978; Trainer et al., 1997).

2,4-D as the results indicate is as equally toxic as pyrethrin with references to brain. Vacuolation in neuroglia, hypergranulation in cytoplasm, neuropicnosis in the nuclei of pyramidal cells of hippocampus gyri and vacuolation or picnosis in the Purkinje cells of cerebellum cortex strongly indicate its toxicity. The single cell like astrocytes, oligodendrocytes in different region of brain exhibit the toxic effect of 2,4-D, which ones upon a time were considered to be non-toxic to them. Koestner (1986) even has gone long way to prove it as brain carcinogenic in rats. Not only the degeneration of neurocytes of different parts of brain he also reported benign tumors in cerebellum after 2,4-D treatment. Paraquat, another herbicide causes pathological changes the large area of brain and necrosis of neurocytes (Bagetta et al., 1992). The observations of Brown (1993); Ferri et al. (2003) again point the 2,4-D toxicity in limbic motor region and damage to the brain.

Neurocytes of medulla oblongata located in upper motor portion are found degenerated when exposed to 2,4-D for a long time. The lack of
movement or the visible paralysis is because of the degeneration of the neurocytes, vacuolation in white mater and irregular distribution of neurons affect the limb movement. In this study, the nuclei from diencephalons and the tract between cerebellum and cerebral hemisphere are showing irregular deposition of neurons. Astrocytes in mesencephalic nuclei and in hippocampus were also different after two 2,4-D doses. The toxicity of the compound causes neuronal changes and astrogliosis (Garcia et al., 2001). Studied in detail about the neurotoxicity of 2,4-D they observed maximum changes in cerebellar cortex and spinal cord.

Groevic et al. (1977); Grant (1980) observed neuropathological changes in the cerebral hemisphere of rat. Hughes (1988); Bagetta et al. (1992) observed damage in the brain and lesions in limbic motor region. 2,4-D toxicity in hippocampus and cerebellum is evident by the destruction of cells. These cells first change their shape, followed by various change in cytoplasm and nucleus as described earlier. These results are similar to those reported by Brusco et al. (1997). Rosso et al. (1997) who observed that 2,4-D at very low concentration is responsible for biochemical changes, producing a plasticity response in gangliocytes contents and composition.

Bortolozzi et al. (1998); Bortolozzi et al. (2001); Bortolozzi et al. (2002) observed many behavioral changes with 2,4-D in rats and damnation in 5-hydroxy tryptamine and dopamine level. They indicate that 2,4-D
induces a regionally specific neurotoxicity in the basal ganglia of rats. Lakshmana and Raju (1996); Unkila et al. (1993) found increase in noradrenalin, dopamine and 5-hydroxy tryptamine in olfactory bulb, hippocampus, visual cortex, cerebellum and brain stem with 2,4-D treatment up to 10 days. After 20 and 25 days the levels of all these compounds in various parts of the brain are reduced. They also reported reduced acetylcholine esterase activity in cerebellum and brain stem.

Kim et al. (1994) and Kim et al. (1995) located 2,4-D in almost every part of the brain, which shows the affinity of this herbicide with the brain tissues and also confirm that such herbicides can easily cross blood-brain barrier and having access to the various part of the brain through CSF also. Bradberry et al. (2000) detected that blood-brain barrier is damaged with 2,4-D so that its amount gradually increases in brain tissues. Accumulation of homovanillic acid and 5-hydroxy 3-indole acetic acid and metabolites of dopamine and serotonin in brain is because of disturbance in the metabolism these neurotransmitters.

4.4.2-pancreas (mixed gland):

In the pyrethrin and fernoxone treated rats no pathological changes could be observed either in exocrine acinar cells or endocrineislet of Langerhan’s. Normal distribution of zymogen granules in pyramidal cells was observed. In β cells uniform distribution of granules in little
concentration, of the central part of cell was seen (Fig- 3.15 and 3.16). Inter acinar alveolar tissue is normal with usual blood vessels and fiber distribution. After 120 days, small vacuolization developed in the cytoplasm of acinar cells, which gradually became large up to the last day of the experiment. Perhaps, due to vacuolization and accumulation of water, cell seems to be in hypertrophy. In β cells, the granules are comparatively low in number and located near the plasma membrane. In the β cells, though scanty number of granules was observed but no vacuolation could be detected. At some places hypertrophy of the lobules was detected (Fig- 3.18).

In some animals number of β cells and islet of langerhans was drastically reduced with the compounds of these classes. The results with pyrethrin or fernoxone about glucose give a different picture. Serum glucose level was estimated high in case of pyrethrin treated rats but irregular pattern of serum glucose level i.e. high-low-high was recorded with fernoxone. It is intriguing to establish the correlationship in the histopathological findings and with quantitative analysis of serum glucose level. It looks like that these compound do not affect β cells up to a long time. Hence, a normal insulin or hyperinsulin level for some times may be maintained in the blood. Hyperinsulinemia can be correlated with fernoxone toxicity, where it disturbs serum glucose level. In both the cases (fernoxone and pyrethrin) after 120 days, fat found to be scattered between
the β cells and in the core of islets of Langerhans. Low level of picnotic nuclei were observed after 120 days treatment, in β cells, which, gradually increased and distinct pynotic nuclei could be seen after 150 days. It seems to be a defensive function of pancreas to accumulate fat to neutralize the toxicity of the compounds. The accumulation of brown fat was found to be gradually increasing. This can be thus, speculated that upon very long exposure to these toxicants a condition may arrive when fat accumulation may occupy a good part in and around islets of Langerhan’s (Fig- 3.17 and 3.19).

After 120 days, the diameter of blood vessels and the cells volume is also found increased. A chronic exposure perhaps leads to pancreatitis. In interlobular or acinar ducts normal cuboidal epithelium is replaced by irregularly shape cells, which, is an indication of hyperplasia.

The histopathological study in relation to these compound have not been much worked out by other investigations. Rozman et al. (1986) found intercapsular changes in the pancreas of rats. Gorski and Rozman (1987) and Liu et al. (1992) found TCDD intoxicated rats are hypersensitive to insulin. There are observations, which indicate clearly that TCDD a chlorinated herbicide causes hypoglycemia and hypoinsulinemia. Silkworth et al. (1982); Ebner et al. (1988) recorded significant decrease in serum insulin level and this change was not associated with changes in serum glucose. Endocrine status of TCDD treated rats depicts decrease in T3, T4,
TSH and insulin level. They recorded no change in glycogen. Another chlorophenoxyacetic acid a derivation of fenoxone is made responsible for increased incidences of non-neoplastic changes in the exocrine pancreas. Nyska et al. (2004) also observed arthritis and lower insulin level in blood. In 2000, Marry and Rosendale indicated severe pancreatitis as concluded by increased pancreatic enzymes activity in blood with herbicides. Inhibition of normal β cells and moderate defused atrophy of pancreatic lobules were reported by Cranmer et al. (2000) and Guo et al. (2003).

4.4.3-Thyroid Gland:

In control, normal epithelium with vascular basement membrane is distinct. Anastomoses and venous plexes associated with the capsule are clear and extensive (Fig. 3.20 to 3.23). The parenchymal tissue of the gland is surrounded by lymphatic capillary flexuses. Capillaries at places are interspersed between the follicular cells. Lobules are irregularly distributed. The single layer of epithelium cells is surrounding the central mass of gelatinous colloidal material, which is stained darkly in many sections. The follicular epithelium cells rest upon thin basal lamina and vary in height from cuboidal to columnar. The variations are greater in such cells. Besides the principal cells, some cells are resting upon epithelial cells are termed as parafollicular cell. Many cysts like structures are interspersed among them. Colloid besides, the containing thyroglobulin, iodine, and protein bound iodine is having enzymes and thyroxine hormone. Principal cells are
responsible for the synthesis of the thyroglobulin, iodination, storage resorption and hydrolysis of thyroglobulin. These are the cells, which, finally synthesize and release T₃ and T₄ in the capillaries or lymph vessels. In cells, whether follicular are principal, many lysosomes and multivasicular bodies are present. Some parafollicular cells are scattered in connective tissue also. Florsheim and Velcoff (1962) observed no histopathological changes in the gland cells. The cytomatic index according to them is found to be variable with 2,4-D treatment. No significant, elevation of TSH and PBI (protein-bound iodine) was reported by them.

Although in some rats low level of TSH was detected in serum but this cannot be taken as a severity of the herbicides, but many researcher like Nakanishi et al. (1970); Potter et al. (1983); Hill et al. (1987); Kannan et al. (1988); Saify et al. (1995) gave a different picture of 2,4-D and pyrethrin. Florsheim et al. (1963) observed the lowering of PBI in serum and this affects to cause the low synthesis of thyroxine. 2,4-D affects the liver, which is the main site of the serum proteins synthesis hence; the proteins responsible to bind iodine in blood could not afford normal iodine level for binding. The results related to glucose in the present study also point to the release of thyroxine. The low level can thus be corroborated with the iodination of thyroglobulin and low metabolic rate, which inhibits glucose absorption.
In 2,4-D treated rats, the variation in the size of the cell is not much studied. These two observation can be correlated with the fact that 2,4-D does not apparently affect thyroid gland. In 2,4-D treated rats almost normal histological structure of thyroid is observed. The amount of the colloid is not much affected. Cuboidal squamous cells are though high in number yet it will be difficult to say that the variation is because of 2,4-D. Roth et al. (1988) did not find 2,4-D as thyromimetic compound using various instrumental detection techniques for T₃, T₄ and TSH in normal and 2,4-D treated rats. They concluded that T₃ level lowers down when herbicides are used in higher concentration. It can be opined that if the T₃ and T₄ level lowers down, this would affect the serum protein synthesis and the toxicity of the compound would aggravate. Because of the serum protein binds with 2,4-D also causing to decrease its toxicity.

Another point is that 2,4-D as reported earlier, affects the different parts of brain including hypothalamus. It can be presumed that the thyrotrophic releasing hormone or inhibitory hormones may be affected. Its not clear whether thyrotrophic releasing hormone decreases or inhibitory hormone increases. Bruner et al. (1996) though did not find 2,4-D compounds as carcinogenic but they supported the view of Van Birgelen et al. (1995) that both, increase in the metabolism and excretion of thyroid hormone take place after the induction from 2,4-D, TCDD or PCB.
Burter and Klaassen (1992); Seo et al. (1995); Osius et al. (1999) observed that chlorophenoxy herbicides like as 2,4-D, TCDD and PCB accentuate binding of the hormone in the liver and T₄ was more actively, joining the plasma membrane of hepatocytes than T₃. In this study, negligible to no damage to the follicular cells could be observed. Though Rickenbacher et al. (1986) and Byrne et al. (1987) found damage to the follicular cells of thyroid. Obviously they reported decreased thyroxine level. In such condition by negative feedback the decreased circulating thyroid hormone would stimulate the pituitary to increase the out put of TSH. This will cause hypertrophy of thyroid, which is also observed in this study. If, the quantitative analysis of these hormones could have been done; a more authentic explanation could be given. One of the herbicides isoxaflutole has been found to induced thyroid tumors in male rats through the disruption in the thyroid feed back mechanism US EPA (1998). The very high concentration of herbicide is found to cause hypertrophy of thyroid with depletion of colloid. This study is done with sub lethal dose under 20 mg/kg body weight hence not much changes related to thyroid follicle epithelial cells etc. were observed.

Gil'manov et al. (1997) found moderate depletion of cellular contents of follicular cells with 120mg 2,4-D in rats. They did not found any tumor incidence with 2,4-D according to them “not even any suggestion of carcinogenicity after 2,4-D administration was noted”. They reported weight loss and increased mortality. Jeffries et al. (1995);
Malysheva and Zhavoronkov (1997) observed histopathological lesions in the thyroid gland of female at 100mg/kg/day. In a histological evaluation of thyroid, the PCB 118 pups revealed changes which is suggestive of sustained TSH stimulation, including increased follicular cell vacuolization, increased nuclear vesiculation, and decreased colloid area (Ness et al., 1993). Kuriyama et al. (2003) found that the perinatal exposure to a low dose of PCB 118 permanently disrupted the hypothalamo-pituitary-thyroid (HPT) axis leading to a significant increase in thyroxine levels in offspring. A ‘thyroid resistance syndrome’ followed by decrease in thyroxine and TSH levels were observed in dams at the end of lactation. 2,4-D administration depletes secretory material in follicular epithelial cells. It can be concluded that lower concentration of 2,4-D for longer time does not affect the thyroid gland much adversely, although changes in epithelium do take places.

Pyrethrin 10mg/kg per body weight for 150 days administered to male and female rats revealed follicular cells hypertrophy (Fig- 3.32 and 3.33). Some squamous cells and less granulated cuboidal cells were noted at places in irregularly distributed follicles. The colloid stained lightly and no vacuolization in the cell or the nucleus was observed. No lesion and hyperplasia could be detected in the thyroid tissues. In thyroid follicular cells hyperplasia was observed after 120 days at a few places (Fig- 3.28 and 3.29), and also reduction in colloid contents. 200 and 500mg/kg body weight caused papillary changes and hypertrophy in epithelial of thyroid. The increase in weight of thyroid was also noticed (Nakanishi et al., 1970).
Saify (1995) observed decrease concentration of $T_3$, $T_4$ and TSH with pyrethrin. The thyroid follicle developed hyperplasia and increased pigment deposition in the colloid of pyrethrin (500mg/kg) treated rats (Graham, 1987). In 2004, one the reports of federal register states that during inhalation of subchronic concentration of pyrethrin, follicular cells hypertrophy was observed in mice. It has not been found to be carcinogenic.

Hard (1998) and a report of 2002 by Nordic Council of Ministers a mild degree of follicular cell hypertrophy was observed in the histological examination. This causes a slight enlargement in the size and increase in the weight of the thyroid gland. The results presented here are in accordance with the observation of various scientists. In one of the reports of UK Health and Safety Executive in 1977, it was observed that 200ppm of pyrethroid for 52 weeks causes thyroid follicular cells hyperplasia but Cabral et al. (1990) observed increased incidences of thyroid tumors with deltamethrin at 8mg/kg body weight for 2 years. It was very difficult for them to correlate high and low doses of cypermethrin with thyroid tumors incidences. Pyrethrins are classified as “likely to be human carcinogens” by Caroline (2002). In this study, no such tumors could be detected in thyroid even after 150 day.
4.4.4-Adrenal Gland:(Fig- 3.36 to 3.47)

Hyperplasia in adrenal medullary cells was clear. At few places, in the cortex moderate to severe fatty degeneration was noticed. Proliferative lesions in the adrenal medulla were also located. The cells of Zona fasciculata have extra granulation in their cytoplasm. These observations vary from the results with other herbicide. Though, 2,4-D has been reported by Hansen et al. (1971) for not to cause gross microscopically lesions in adrenal cortex. According to them small haemangeoma were seen in the cortex of the adrenal gland.

DiBartolomeis et al. (1987) correlated the concentration of steroids level in serum with the histology of the adrenal gland. And slight increase in cholesterol content in the cell for its incorporation in steroidogenesis. They also recorded swelling in the mitochondria because of increased synthesis of steroid. Gorski et al. (1988-a) found the relationship in the hypertrophy of the adrenal cortex; increase serum steroid level and toxic stress of the TCDD. Histopathological results given here clearly point out that chronic exposure of pyrethroid finally cause vacuolization in the cytoplasm, hypertrophy of the cortical region and ultimately lesions at places. Mohemed et al. (1985) observed accumulation of radioactive pyrethroid in the zona fasciculata and at high dose in zona glomarulosa too. The invitro studies revealed the inhibition of ACTH by toxophene. Through the experiments they pointed out that the chronic exposure is adenotoxic.
The treatment of herbicide gives even serious picture of cell pathology of the adrenal gland. Large vacuolation in the cytoplasm, picnosis of nuclei, degeneration of mitochondria, deposition of fat and atrophy of cells of adrenal cortex and chromofin cells are observed. Here not only vacuolation of zona reticularis and glomarulosa is seen, but disturbed pattern of cells was also marked. TCDD, a type of chlorophenoxy herbicide produces almost similar effects in adrenal gland. Thymus gland in treated rats was found regressed completely and adrenal gland was severely damaged (Gorski et al., 1988-b). As it is said earlier the adrenal gland counteract the toxic effect of pyrethrin or 2,4-D up to a level. It is evident by the finding that a sublethal dose of 2,4-D or TCDD may be proved lethal to adenolactomised rat. If such rats are given cortisones/corticoids their survival chances increase. Charles et al. (1996) studied the effect of 2,4-D on many organs of the rats including adrenal gland. Hypertrophy was reported in the cells of zona glomarulosa of adrenal cortex and while in female, a slight enlargement of adrenal gland was observed. 2,4-D at 90 days exposure to rats alters circadian rhythm in the adrenal and thyroid glands (Nicolau, 1983).

The present study clearly points to the fact that the compounds pyrethrins as well as fenoxone are harmful to mammals, the toxicity of pyrethrin in comparison to fenoxone is high. Brain among the organ studied is the most sensitive, and is affected much with variable concentration of these two different groups of chemicals. The development of lesions, atrophy of Purkinje cells, and possibly disturbances in the relay
system of different part of brain is exhibited by the apparent change in the behaviour. Though, the histological study of liver is not given here but the enzymes parameters clearly point out severe damage by the compounds to liver. Regioning of muscles, lethargy, and slackness of the limbs are the few observations among many, which indicate that the chronic exposure to these compounds lead to muscular debility. The carcinogenicity of the compounds have been taken into consideration but no such growth could be detected undoubtedly, harmful effects on liver, brain, and some endocrine glands are the given here.

The use of fernoxone may be necessary for the eradication of the herbs good yield of crop. Fernoxone is being used by villagers in there fields because of unavailability of labours and more on financial grounds, pyrethrin is used both inside and, out of the house as insecticide to meet the menace of mosquitoes but both of them are hazardous to human being. The quantity of pyrethrins used in the houses though is very less yet used almost regularly in houses to get rid of malaria infestation and itching sensation of mosquito bite. Its regular use will in long-term be harmful to human population. More ever, use of pyrethrin and fernoxone in a field leave long-term negative effects in multiple ways to our ecosystems and affecting living being including man. Hence their use is either be banned or an awakening be developed for their judicious use.