Effect of herbicides on general behavior of treated animals viz. locomotion, eating and drinking habits, timidness, aggressiveness, jumping habit etc. has been observed. Rosso et al. (2000b), reported loss of muscular strength, righting reflexes and mode of coordination. Some of the above behavior are dose dependant of fernoxone and butoxone which aggravate them. Continuous hiccups and drinking are the initial responses which animal shows for 12 to 16 hours upon feeding the herbicide (Hundal et al., 1998). The animal regains its normal attitude after 16 hours also. After 35 days of continuous treatment the running, jumping and movement towards food and water sources started declining (Iyaniwura and Okonkwo, 2004). It is also observed sometimes that some of the treated rats rub their front legs so vigorously with their face that it even bleeds them (Saxena and Sharma, 2000).

Fernoxone and butoxone over-activate the cholinergic and dopamine receptors present in the cerebral cortex (Mattson et al., 1997; Tuschl et al., 2003; Lazarini et al., 2001). The over activation of the receptors led to such abnormality in behavior. Bortolozzi et al. (2003); Laxmana et al. (1996), observed the increased level of catecholamine with lower doses of herbicides given for 25 days. They also speculated that the cynocompounds formed after metabolism of the herbicides affecting the concentration of neurotransmitters and thereby the activities of the receptors. Hervonen et al. (1982);
Gupta et al. (1999) explained the damage to the blood-brain barrier. Acute and chronic dietary neurotoxicity was reported by Mattson et al. (1997).

Gilad et al. (1992), explained their results on the basis of synaptic transmission and change in the binding characteristic of various neurotransmitters receptor-ionophore complexed in rat brain. The influence of cyno group on adrenal medulla and basal ganglion of the brain release more amount of neurotransmitters which also disturb \( \text{Na}^+ \) gating. \( \text{Na}^+ \) channels also play an important role in the release of catecholamines and adrenalin from nerve terminals (Eells et al., 1992) showed that the prolonged effects of herbicides proved to be deleterious because in the central nervous system (cerebellum, hypothalamus and cerebrum) unmetabolized herbicides accumulate to disturb the normal functioning of neurons.

Loss of appetite and loss of weight of animals can be correlated with damaged liver and impaired thyroid function. The inarticulate movement in these animals is collaborated with the hypofunction of thyroid gland in treated animals. DeLima et al. (2008), reported cirrhosis in herbicide treated rats. Hossainain (2012) observed the effect of herbicide on liver function. His histological finding is almost similar to what is reported here. He also observed hyperplasia and necrosis in various parts of the liver. After 2 months of experimentation he was able to show the start of hepatic carcinogenicity. Kalipci and Ozdemir (2011),
did not encounter any carcinoma in the liver of rats with the treatment of 45 mg/kg b.w. herbicide for more than 30 days.

**THYROID GLAND**

In untreated animals, normal epithelium with vascular basement membrane is distinct. Anastomoses and venous plexus associated with the capsule are clear and extensive (Fig 3.1 to 3.5). The parenchymal tissue of the gland is surrounded by lymphatic capillary plexus. Capillaries are interspersed between the follicular cells. Lobules are irregularly distributed. The single layer of epithelial 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. Colloid contains thyroglobulin, iodine and protein bound iodine having enzymes and thyroxine hormone. Main cells are responsible for the synthesis of the thyroglobulin, iodination, storage resorption and hydrolysis of thyroglobulin. These cells release T₃ and T₄ in the capillaries or lymph vessels. In main cells many lysosomes and multivascular bodies are present. Some parafollicular cells are scattered in connective tissue also.

In treated rats T₃ and T₄ level lowers down when herbicides are used in higher concentration. If the T₃ and T₄ level lowers down it
affects the serum protein synthesis and the toxicity of the compound increases. Florsheim et al. (1963) and Floi-Mlicin et al. (1962) found damage to the follicular cells of thyroid. They found decreased thyroxine level. In this way the decreased level of circulating thyroid hormone stimulate the pituitary to increase the output of TSH. This causes hypertrophy of thyroid which is also observed in this study. The herbicides isoxaflutole has been found to induce thyroid tumors in male rats through the disruption in the thyroid feedback mechanism US EPA (1996). The very high concentration of herbicide is found to cause hypertrophy of thyroid with depletion of colloid. Jefferies et al. (1995); Malysheva and Zhavoronkov (1997) found histopathological lesions in the thyroid gland of female at 100 mg/kg/day. A ‘thyroid resistance syndrome’ followed by decrease in thyroxine and TSH levels were observed in rats. 2,4-D administration depletes secretary material in follicular epithelial cells.

2,4-DB 10 mg/kg per body weight for 145 days administered to both male and female rats revealed follicular cells hypertrophy (Fig. 3.3). In thyroid follicular cells hyperplasia was observed after 115 days at a few places (Fig. 3.2) and also reduction in colloid contents. The increase in weight of thyroid was also noticed. In 2004, one of the reports of federal registrar 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 (2002) 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. Cabral et al. (1990) found that the increased incidences of thyroid tumors with deltamethrin at 8 mg/kg b.w for 2 years. In this study no such tumors could be detected in thyroid even after 145 days.

**PANCREAS**

The results of this work are again confirmed by increased level of SGOT, SGPT and LDH. Gorzinski et al. (1987) and Timchalk (2004) observed changes in haematological parameters, increased level of SGOT/ALT were reported by them in treated animals. Sharma et al. (2005) induced low level of antioxidants status in liver in herbicides exposed animal. Oztas (2000) found early stages of pancreatic carcinogenesis in rat pancreas, only minor pathological changes could be observed both in exocrine acinar cells and endocrine islets of Langehan’s. The duct cells showed hypotrophy can be interpreted with less watery secretion in pancreatic juice and causing concentrated juice flow in duodenum. Sparsely distributed zymogen granule in pyramidal cells is indication of negative effect of herbicide. β cells in pancreas showed progressive degeneration with dose and days. Obviously, deficiency of insulin will elevate serum glucose level, Bradberry et al. (2000); Kishnappa et al. (2000) and Pushpalatha et al.
(2010) have reported high glucose serum level, but no conclusive suggestion was given by them for hyperglycemia. After 115 days small vacuolization developed in cytoplasm of acinar cells which gradually became larger up to the last day of the experiment. In β cells, the granules are comparatively low in number and located near the plasma membrane, no vacuolization or hypertrophy could be observed. In some animals the different type of cells are found in reduced size and number adipose cells get accumulated in the core of islets (Rozman et al., 1986). Picnotic nuclei in β cells were observed after 145 days. The prolonged treatment increased diameter of blood vessels and volume of some cuboidal epithelial cells was found to increase.

**LIVER**

Moderate to excessive changes in the hepatocytes and in the texture of liver were observed with the days of treatment passed-on and the concentration of the herbicides became higher. In histological sections blood congestion, damage to the centro-lobular vein and necrosis of the hepatocytes were observed. Larger gaps between the hepatic cards and endothelium show the retention of fluid. Pycnotic nuclei, vacuolation of cytoplasm hypertrophy of kupffer cells and infiltration of lymphocytes in liver tissue are some of the detrimental effects of the herbicides. The increase in SGOT and SGPT level in both cases support the histological findings. Ahmed (1986); Gupta (1988);
Tawill and Rehman (2001) also suggested that the cytotoxic effect of the herbicide. A significant argumentation of the level of the SGOT and SGPT was observed due to hepatotoxic effect of herbicides by Yamano et al. (1992) and Nanda et al. (1996) noticed a progressive increase in the level of SGOT and SGPT which they co-related with liver damage because of the herbicide. Khogali et al. (2005), also reported the effect of dimathoate – a herbicide, on liver histology. Treatment of herbicides increased LDH in the blood serum of the experimental animal. It was observed that a decrease in oxygen consumption occurred in hepatocytes with an increase in concentration of herbicide. Sharma et al. (2005) observed the effect of cypermethrin on LDH activity. They observed the toxic effect of the compound on liver, kidney, haematocrit value and serum protein pattern. Their findings regarding histopathological changes in liver like destruction of liver nuclei, infiltration of lymphocytes and hypertrophy of kupffer cells are in accordance with Sharkoori et al. (1990) and Thangavel et al. (1994). Thangavel et al. studied the effect of the herbicide on a teleost fish S. mossambicus. Abdu Rabou (1996) and Shakoori et al. (1992) observed the similar results of the herbicides on animals. Recently, Hassanein et al. (2012) reported the hepatotoxic effect of the herbicide and correlated the toxicity with carcinogenicity. He emphatically stressed the compounds as carcinogenic. He referred Bannasch and Zerban (1997); Spencer
et al. (2002) and Newton et al. (2001) who observed spongiosis hepatitis an indicator of hepatocarcinogenicity.

McClintock and Gollopudi (1990) and Jefries et al. (1995) observed a gradually increased region in the liver of herbicide treated mice, no carcinogenic structure was observed by them. Kalpci and Ozdemir (2010), did not find any significant change in the weight of the liver in treated and untreated animals. Hydrotropic degeneration in the parenchyma of the peripheral tissues of liver was observed by them. Hypertrophy leading to necrosis and damage of hepatocytes around central lobular was prominently observed as one of the effect of herbicide (Palmeira et al., 1994). Dilatation of blood sinusoids and proliferation of kupffer cells is a common phenomena in treated animals. After 145 days similarly Konjuh et al. (2008) reported depletion of fibrous layer of Gilsson’s capsule and hypomyelanation caused by the treatment of herbicides.

**TESTES**

In the present study it is observed that the administration of 2,4-D also changes the biochemical parameters of the reproductive tract. In acute testicular toxicity the reduction in the weight of the testes may be due to decreased number of germ cells and elongated spermatids. 2,4,-D reduce spermatogenic potential by reducing the number of Sertoli and spermatogonial cells, as weight of testis is largely dependent on the mass of differentiated spermatogenic cells (Charles
et al., 2001). It may be due to reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of Leydig cells, a site of steroid biosynthesis (Sujatha et al., 2001). Similarly due to low bio-availability of androgen the weight of accessory sex organ also decrease as well are androgen dependent depleted androgen concentration might cause reduction in weight (Ellis et al., 1998). 2,4-D exposure is associated with low-sperm count and under weight male sex organs in laboratory studies. US EPA (2004) reports atrophy of the testes, degeneration of sperm-producing tissues and decreased numbers of sperm in the testes. National research Centre (Egypt) finds that 2,4-D also caused an increase numbers of abnormal sperm. 2,4-D also increases the level of sex hormone (Garry et al., 2001). Degeneration of seminiferous tubules and lumenae of the epididymis were mostly free from spermatozoa (Jewell et al., 1998).

After 75 days of treatment of fernoxone and butoxone condensation of blood vessels were observed. Narrowing of seminiferous tubules had gradually started, Necrosis and sluffing of seminiferous tubules (germinal epithelium) was evident as the results of herbicides, monocular lymphocytes infiltration was observed at many places. Necrobiotic changes were observed in the tissue sections. The reduced number of spermatogonia and thining of sperm bundles were the dose dependent effects of the herbicides (Oakes et al., 2002 and Salem et al., 1998). The results of the present study confirm the findings of Hossainain (2012). Konjuh et al. (2008) who found necrosis in
spermatogenic tubules, infiltration of lymphocytes in epididymis in 2,4-D treated rats. Server et al. (1997) found that 2,4-D reduced sperm count and increased abnormalities in human sperms. In 2004, Tmchark also reported similar effects with reference to testes in 2,4-D treated dogs. Lipid hydroperoxide and ascorbic acid were elaborated with T₃ treatment in adult rat testes. T₃ is also a type of herbicide commonly used to eradicate monocot wheat from the agricultural fields. They investigated that herbicides mainly affect a glutathione redox pool and mitochondrial enzymes. Mitochondria and enzymes are also associated with glutathione redox too. In this case percentage of dead sperms gradually increased as the time of treatment with herbicide is enlengthened (Joshi et al., 2007, 2012). It cannot be overruled that hydrogen peroxide generated in response to the toxicity of herbicide probably, first affect the leydig cells. Peroxidation in the presence of free radical augment, damage to testes, testosterone and reducing serum level of testosterone (Ellis, 1998; Reddy et al., 2006). The decreased level of the steroid hormone and a negative effect on Sertoli cells might be responsible for increased percentage of sperm death. The toxic effect of the herbicide on liver and thyroid became associated factors for lowering of sperm count, degeneration of germinal epithelial cells and increased motility of sperm. Choudhary et al. (2003) correlated the influence of hypothyroidism on antioxidant defense system in adult rat testis. Although Mairino et al. (1998) did not notice any change in GPX₁ activity yet they found the
hexachlorocyclohexane induced oxidative stress in testis. The similar effects were also reported by Penitola et al. (1996). One thing is interesting that during 75 to 115 days of treatment, number of mitochondria increased in the cells of germinal epithelial this is perhaps, to meet the toxic effect and to neutralize them by glutathion redox pool. This pool reduces free oxygen radicals. 2,4-D, a herbicide causes slide decrease in testosterone release and significant increase in estrogen release from testicular cells (Lieu et al., 1996). Male farm sprayers exposed to herbicide had lower sperm count and more spermatic abnormalities as compared to man who were not exposed to the herbicides (Lerda and Rizzi, 1991).

**OVARY**

New studies indicates that 2,4-D reduces fertility in several ways. Scanty literature was available in relation to the effect of herbicide on the mammalian ovary. As describe earlier (see results on ovary). It is obvious from the histological findings that the herbicides hampor the formation of follicles and also caused the abnormalities in the follicular development. As compared to control herbicide treated animals had fewer to negligible number of carpus luteum in their ovaries. Number of atritic follicles was increased. Degeneration in the germinal epithelium of ovary was observed at many places. Besides these changes other toxic changes affected the fertility of female rats. Rawling et al. (1998) observed that the herbicides affected the serum estrogen and other steroids in female. These two hormone are related
with the development of ova in ovary and preparation of female reproductory tracts for conception. Obviously, the lowered concentration of these female steroid hormones affected the formation of ova and fertility of the animal. Abnormal sister chromatid exchange in somatin in germ cell of mice exposed to 2,4-D, a herbicide caused abnormal ova formation. Rawling et al. (1998) also observed abnormalities in reproductive endocrine system in eves. Sharkoori et al. (1990) did not see any teratogenic effect in treated animals. In rodents, herbicide treatment increased the level of the hormone progesterone and prolactin and caused the abnormality in estrous cycle (Duffard, 1995). Garry et al. (1996) observed increase in birth defects in herbicide exposed animals. Innocent intake of the new toxins are alarmingly increasing the human problem in multiple ways - Female infertility, formation of defective ova and abnormal embryonic development seem to be direct effects on ovary and tubular female reproductive parts. When herbicides were fed to pregnant female rats average litter size was reduced by about 20%. National Institute for Occupation Safety and Health identified in laboratory tests that sprays of aromatic solvent-Nephtha reduced fertility and reduced the growth of new borns. Abnormal foetal skeletal development, increased foetal mortality and other reproductive effects are fairly conclusively associated with exposure to phenoxyacid herbicide and their dioxin contaminants (IPCs, Geneva 1984; Hurst et al., 2002). Reproductive toxicity has also been observed in relation
to herbicides. In rats herbicide exposure resulted in foetuses with abnormal cavity bleeding, increased mortality and genetic damage (ETN, 1996).

The present study clearly indicates that both the herbicides fernoxone and butaxone are higher damaging. Liver, pancreas and the other organs are affected much with various concentrations of these two phenoxy groups of herbicides.