Histopathology

Histology is the study of microanatomy of specific tissues and it has been successfully employed as a diagnostic tool in medical and veterinary sciences since the first cellular investigations carried out in the 19th century. Histology in a precise sense, is the study of the cytoarchitectural change of the body, which envisage the anatomy and gives the insight into the functioning of tissues and organs (Madhava Rao et al., 2009). Histology is the best and most direct method of studying xenobiotics by means of in vivo scanning and photographic presentation. The study and examination of normal cells and tissues by microscopy is called histology or microscopic anatomy. The study of abnormal cells and tissues is histopathology (Aughey and Frye, 2001).

Histology is the study of tissue sectioned as a thin slice, using a microtome. Histopathology is the microscopic study of diseased tissue and is an important tool of anatomical pathology since accurate diagnosis of cancer and other diseases usually requires histopathological examination of samples. The trained scientists who perform the preparation of histological sections are known as histotechnicians, histology technicians (HT), histology technologists (HTL), and medical scientists, medical laboratory technicians or biomedical scientists. Their field of study is called Histotechnology (Merck Source, 2002 and Sted man’s medical dictionaries, 2005).

The examination and study of normal cells and tissues by microscopy is called histology or microscopic anatomy. The study of abnormal cells and tissues is histopathology (Aughey and Frye, 2001). Toxicological histopathology gives useful data concerning the changes induced by chemicals at the tissue and cellular level. All the tissues and organs in the body of an animal may be potential targets for the toxic effects of any chemical or metal. A histopathological assessment throws light on the nature of tissue alteration and the extent of damage. This in turn indicates the toxic nature of the compound. Therefore, histology gives useful insight in to the tissue lesions prove to the external manifestations of the deleterious effects of heavy metals or any chemical.
Toxic effect of OP compounds on nervous tissue: Neurotoxic effects of chemical and drugs in experimental animals have been evaluated by a number of approaches including clinical assessment, neurotoxicity neurophysiology and neuropathology. The neuropathology, comprising microscopically assessed morphological and immuno histochemical changes, is a foundation of neurotoxicology and has long been employed to enhance the understanding of chemical injury to the nervous system. In addition regulatory agencies require detailed neuropathologic evaluation in animal studies as part of their assessment of chemical agents to ensure the public safety (OECD 2003). Pesticides which are ubiquitous in nature have become integral part in the tissues of animals. Pesticides find their way into places far from application and accumulate in significant concentration in the tissues of animals.

Pesticide residues in the tissue cause serious physiological alterations even at low levels after a prolonged period of exposure to chemical compounds. It is obvious that any chemical insult could cause pathological damage injury to cells in an animal. The severity of tissue damage depends on the concentration and potentiality of toxic compound accumulated in tissues and it is time dependent. The cellular and sub-cellular constituents of tissue in terms of size, shape, number and position play an important role in the physiological and metabolic function. Therefore, the histological structure of tissue in an animal has a profound influence on its function. Physiological studies alone do not satisfy the complete understanding of pathological conditions alone of tissues under toxic stress. Hence, it is useful to analyze the histological aspects. The extent of the severity of the tissues damage is a consequence of the concentration of the toxicant and is the time dependent. Moreover, the severity of damage depends on the toxic potentiality of a particular compound or pesticide accumulated in the tissue. Several studies have demonstrated that pesticides such as organochlorines, OPs, carbamates and pyrethroids produced embryo toxicity, genotoxicity, teratogenecity and tissue damage (Cavas and Ergene 2003; Soni and Bhatnagar, 2005; Tisch et al., 2005).

Several workers reported that the pesticides cause architectural damage in brain, gill, liver, kidney, heart, lung, muscle, testis, intestine in various animals (Shukla et al., 2001, Glynn, 2003; Garg et al., 2004; Madhaveelatha, 2006; Sivaiah, 2006; Rajendra Prasad,
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2007; Sukanya, 2007; Kishandar, 2007; Venkatachandrudu and Radhakrishnaih, 2008; Rajeswari 2008; Appa Rao et al., 2009; Hooser and Earnes, 2010; Aswini, 2010; Suseela 2011; Prasanna Lakshmi 2012; Siva Prasad 2013; Devisrilakshmi kala 2014). As histopathology is a critical part of the toxicologic and risk assessment of food, drugs and chemicals, it is important that the approach to histopathological examination satisfy both the regulatory demands for unbiased observations while facilitating the sensitive and efficient evaluation of large amount of microscopic information (James et al., 2004).

In view of the above, an attempt had been made to study the structural changes in brain regions of Albino rat exposed to sublethal dose of Profenofos.

Results

The tissues like Cerebral cortex, Hippocampus, Cerebellum, Medulla oblongata and Thyroid gland treated with Profenofos are studied by light microscopy and compared with that of control brain regions and Thyroid gland.

Light Microscopy

Normal histology of rat Cerebral cortex

The Cerebral cortex is the largest part of vertebrate and is the source of neural transactions that enhance memory, plasticity, cognition, speech and intellectual activity. The cytoarchitectural structure of cortex is characterized by the presence of six – layered laminated pattern of cells.

1st layer – Consists of mostly glial cells, axons of neurons of other layers and very few neurons.

2nd layer – Small pyramidal cells

3rd layer - Large pyramidal cells

4th layer – Rich with stellate and granule cells which receive input to the cortex from thalamocortical fibers, association fibers and commissural fibers.
5th layer – Largest pyramidal cells known as giant pyramidal cells or Betz cells

6th layer – Martinotti cells.

Microphotograph of Group I (control) rat Cerebral cortex shows different layers such as molecular layer with glial cells (GC), pyramidal cells (PC), Golgi cells (GOL.C) and Neurofibrillar network (NFN) (Fig. 5.1).

**Histology of rat Cerebral cortex under Profenofos toxicity**

The cerebral cortex of Group II rats showed loss of neuronal process (LNP), light congestion of blood vessel (LCBV), pyramidal cells (PC) and mild hemorrhage (M.Hae) (Fig. 5.2). The cortex of Group III rats showed the loss of neuronal process (LNP), mild congestion of blood vessel (MCBV), mild (M.Hae) and vacuolation (V) (Fig. 5.3). The cortex of Group IV rats showed remarkable damage in the cytoarchitectural changes, which included pyknosis of neurons (Pyc. N), moderate congestion of blood vessel (MCBV), Virchow robins space (VRS) and vacuolation (V) were observed (Fig. 5.4).

**Normal histology rat Hippocampus**

The hippocampus lies under the medial temporal lobe one on each side of brain and is part of limbic system. Hippocampus plays significant role in the formation of long-term memories. Hippocampus is grouped with nearby structures including dentate gyrus and is called hippocampal formation. The hippocampal formation is bilateral structure sandwiched between the cortex and the thalamus. Hippocampal formation consists of hippocampus proper, the dentate gyrus, the subicular complex and the fornix. Hippocampus proper is subdivided into four main cytoarchitectural fields namely CA1, CA2, CA3 and CA4 that are unidirectional connected from CA4 to CA1. CA2 and CA4 are small and not well defined. The neurons of hippocampus have spatial firing fields called Place cells. Pyramidal cells are present in CA3 and CA1 regions. Some important anatomical features of hippocampus are as follows.
1) Dentate gyrus possesses 1.2 million granule cells, 4K basket cells 32 K hilar inter neurons and 20 K mossy cells.

2) CA3 subfield has 1.6 x 10^3 Pyramidal cells and CA1 consists of 250 x 10^3 Pyramidal cells. Together CA3 / CA1 have 330K / 420K pyramidal cells and various inter neurons.

3) Subiculum possesses around 180 K cells.

4) Hippocampal formation is formed of Enthorhinal cortex, dentate gyrus, CA3 CA1 and Subiculum.

   Microphotograph of Group I (control) rat hippocampus showed characteristic curvature of hippocampus. Cornu Ammonis layers (CA1 and CA3) are separated by compact glial cells (GC), neurons with neuro fibrillary net work (NFN) and Pyramidal cells (PC) are present in CA3 and CA1 region (Fig. 5.5).

**Histology of rat Hippocampus under Profenofos toxicity**

   The cytoarchitectural changes were less prominent in hippocampus of Group II administered rats. The changes in hippocampus showing the vacuolation (V), glial cells (GC), neurons with neuronal processes (N) and loss of neurofibrillar network (LNFN) (Fig. 5.6). Group III treated rat hippocampus showed vacuolation (V) in neuro fibrillar network, dilated blood vessel (DIBV), Loss of architectural details (LAD), slight necrosis (SN), pyramidal cells (PC) and degenerated neuron (DN) (Fig. 5.7). Remarkable changes were observed in Group IV administered rat Hippocampus with the moderate congestion of blood vessel (MCBV), congestion of glial cells (CGC), dilated blood vessel (DIBV), congestion blood vessel (CBV) and loss of architectural details (LAD) are noticed (Fig. 5.8).

**Normal histology of rat Cerebellum**

   One of the most impressive parts of the brain is cerebellum, located at the lower back of the brain and acts more rapidly than any other part of the brain. The cerebellum is not only involved in skilled motor performances but also involved in various sensory functions including sensory acquisition, discrimination, tracking, prediction etc. The three functional regions of cerebellum are vestibule-cerebellum, spino-cerebellum and cerebro-
cerebellum. The cerebellum can be divided into three cortical layers with the same basic neuronal circuitry everywhere which involve five main cell types as follows.

1) Outer Molecular layer - Basket cells and Stellate cells

2) Middle Purkinje layer - Purkinje cells (Largest neurons)

3) Inner Granule layer - Granule cells and Golgi cells

Microphotograph of Group I (control) rat cerebellum showed Neurofibrillar network (NFN), glial cell (GC) and with architectural details (AD) (Fig. 5.9).

**Histology of rat Cerebellum under Profenofos toxicity**

Cerebellum of Group II treated rat showed the vacuolation (V), glial cell (GC), neurons with neuronal processes (N) and Loss of neurofibrillar network (LNFN) (Fig. 5.10). Group III treated rat cerebellum showed vacuolation (V) in neuro fibrillar network, dilated blood vessel (DIBV), Loss of architectural details (LAD), slight necrosis (SN), degenerated neuron (DN) (Fig. 5.11). The cytoarchitectural changes of Group IV treated rat cerebellum showed the moderate congestion of blood vessel (MCBV), congestion of glial cells (CGC), congestion blood vessel (CBV), dilated blood vessel (DIBV) and loss of architectural details (LAD) are noticed (Fig. 5.12).

**Normal histology of rat Medulla Oblongata**

Medulla oblongata is the lowermost portion of the vertebrate brain stem and functions as relay station for the crossing of motor tracts between the spinal cord and the brain. Medulla oblongata is responsible for autonomic functions such as respiration, blood pressure, heart rate, swallowing, vomiting, defecation, gagging, coughing etc. The cross section of Group I (control) rat medulla oblongata showed long axon pyramidal cells (PC), glial cells (GC), neuronal cells (NC) and neurofibrillar network (NFN) (Fig. 5.13).
Histology of rat Medulla Oblongata under Profenofos toxicity

Group II treated rats showed clumping of neuronal cells (CLNC), dilated blood vessel (DIBV), granular cell (GC), neuronal cell (NC), degenerated granular cell (DGC), degenerated neuronal cell (DNC), Vacuolation (V) and excessive vacuolation (EV) (Fig. 5.14). The cytoarchitectural changes in Group III rats showed mild congestion of blood vessel (MCBV), vacuolation (V), pycnotic nucleus (PYN), loss of architectural details (LAD) and dilated blood vessel (DIBV) (Fig. 5.15). The Medulla oblongata Group IV rats showed vacuolation (V), mild congestion blood vessel (MCBV) and loss of neurofibrillar network (LNFN) (Fig. 5.16).

Normal histology of Thyroid gland

The thyroid is composed of spherical follicles. Inside the follicles, in a region called the follicular lumen, colloid serves as a reservoir of materials for thyroid hormone production and, to a lesser extent, acts as a reservoir for the hormones themselves. Colloid is rich in a protein called thyroglobulin. The follicles are surrounded by a single layer of thyroid epithelial cells, which secrete T3 and T4. When the gland is not secreting T3 and T4 (inactive), the epithelial cells range from low columnar to cuboidal cells. When active, the epithelial cells become tall columnar cells. Scattered among follicular cells and in spaces between the spherical follicles is another type of thyroid cell, parafollicular cells, which secrete calcitonin.

Microphotograph of Group I (control) rat Thyroid gland shows the Follicles (F), Follicular lumen (FL), colloid (C) and Follicular Epithilium (FE) (Fig. 5.17).

Histology of rat Thyroid gland under Profenofos toxicity

In Group II administered rats, histopathological changes in the Thyroid gland were less prominent showing Slight vacuolated colloid (SVC), Epithelium cells (EC) and C-Cell (C) (Fig. 5.18). The cytoarchitectural changes in Group III treated rats showed Moderate differentiated Follicles (MDF), Slight hemorrhage Follicular lumen (SHFL) and Disturbed Epithelial cells (DEC) (Fig. 5.19). They thyroid gland of Group IV animals showed the Poorly differentiated Follicles (PDF), Hemorrhage Follicular lumen (HFL) and Vacuolated colloid (VC) (Fig. 5.20).
Discussion

Since the time immemorial man has been fascinated by the beauty and the activity of the brain. It has been challenging to understand the structural and functional anatomy of any organ until the discovery of techniques such as perfusion fixation, plastic embedding, light microscopy and structural examination of the tissues, cells and cell organelles. Of all the tissues, nervous tissues presented extraordinary challenges to scientific world because of its complex anatomy of neurons and fibers. The difficulty in histopathological study of brain lies in complexity of its anatomical structure. While pathological examination of nervous system is an important component of neurotoxicology, the features of the brain can make the assessment challenging. Among these are its cellular complexity, regional variation in structure and function and multiplicity of reaction to injury. In addition there is propensity for histological artifacts to occur in nervous tissue samples unless scrupulous attention is paid to dissection, sampling fixation, processing, sectioning and staining of the material. In order to avoid misinterpretations one should be careful and aware of the nature of artifacts.

The present study has clearly revealed damage in the cytoarchitectural changes in different regions of rat brain of Group II, III and IV animals. The neuro histopathological changes were more pronounced in Group IV days treated rats, when compared to other groups. The observations were in agreement with OP induced neurotoxicity. Histopathological parameters were studied to evaluate the extent of neurotoxicity of Profenofos in time and days dependent manner. The damage to normal cytoarchitecture of cells was observed in almost all regions of brain studied. Severities of changes in different regions of brain are in accordance with the time and days regimen. Severity was highest in Group IV rats and least in Group II rats and Group III treated rats showed intermediary neuropathologic changes. The changes in cell dynamics of Profenofos treated rat cortex showed mild hemorrhage, loss of neuronal processes, Pyramidal cells and vacuolation.

Cytoarchitectural changes in hippocampus of Profenofos treated rats include shows the moderate congestion of blood vessel, congestion of glial cells, dilated blood vessel, loss of archetectural details are noticed. The histopathological changes in cerebellum of Profenofos intoxicated rat showed the moderate congestion of blood vessel, congestion of
glial cell, dilated blood vessel. The loss of architectural details were noticed in the medulla oblongata of rats exposed to sublethal dose of Profenofos showed vacuolation, mild congestion blood vessel, loss of neurofibrillar network. Histopathological changes in Thyroid gland of Profenofos treated rats poorly differentiated Follicles, Hemorrhage Follicular lumen, vacuolatedcolloid

Calson et al., (2000) observed that OP compounds induced cell death in SY-SY 5Y human neuroblastoma cells. The adverse effects of OP’s on brain development reflected the same basic mechanism that underlies systemic toxicity, namely cholinesterases inhibition and consequent cholinergic hyper stimulation (Mileson et al., 1998; Pope 1999). Purohit, (2005) observed mild hemorrhages and fatty changes due to decomposition and metabolism of Acephate in to methamidophos in liver of white leghorn birds. Sushila Patel et al., (2006) reported cypermethrin induced DNA strand breaks in different organs and tissues of Mice, with the brain showing highest level of damage. Mukhopadyay et al., (2004) reported cypermethrin caused DNA damage in brain ganglia of drosophila melanogaster. Abdel – Rahman et al., (2001) reported the daily dermal application of DEET (N,N- diethyl m-toluamide) at 40mg/kg, permethrin at 0.13mg/kg or a combination of both compounds for 60 days produced a diffused neuronal death in the motor cortex, different subfields of the hippocampal formation and the purkinje cell layer of cerebellum.

The daily dermal exposure to malathion or permethrin, or in various combinations for 30 days induced neuronal changes in the hippocampus, brain stem and cerebellum of rats (Abdel – Rahman et al., 2004). Abdel – Rahman et al., (2001) pointed out that some 45% of the neurons in layer III of the motor cortex are dying in rats dermally exposed to 40mg/kg/day DEET for 60 days. Tryphonas and Clement (1995) reported histopathological lesions, hemorrhages, perivascular and pericellular edema in brain, heart, liver and lungs. Neurodegeneration with neuronal death, glial proliferation and neurotransmitter changes have been shown in mouse administered with methamphetamine (Schemued and Bowyer, 1997). Petras (1994) reported that the axon degeneration in cortex, sub thalamic region, hippocampus, fornix’s, septum, preoptic area, superior colliculus, basil arpontine nuclei, medullary tegmentum and cortic spinal tracts of the rat when they were treated with soman (3,3 – dimethyl – 2 – butyl methyl –
phosphoro-flouridate) at dose levels of 79.4 to 114.8mg/kg body weight. Abdel – Rahman et al., (2004) reported that the rats treated with nicotine or chlorpyrifos or combination of nicotine and chlorpyrifos from gestational days to 20 showed significant neurological changes. Sivaiah (2006) reported necrotic changes and drastic reduction in granular layer of cerebellum of monocrotophos treated mice. Latuszynski et al., (1999) reported that the dermal exposure of chlorpyrifos and cypermethrin leads to the several histopathological changes as well as increased density of the cytoplasm in focal pyknosis of the cytoplasm in the cerebral cortex and the cerebellum. Intoxication by soman causes prolonged seizures that lead to neuropathology in the brain (Bhagat et al., 2001). When the Sprague – Dawley rats were administered with tebuconazole, a triazole caused cell loss with in pyramidal cell layer of CA3 – CA4 fields of the hippocampus and layer V of the neo cortex (Moser et al., 1990).

Rajendra Prasad (2007) reported that exposure of low doses chlorpyrifos in rat results in cytoarchitectural changes such as congestion of blood vessels, loss of neuronal process, appearance of vesicular nucleus, reduction in number of purkinje and granule cells, necrosis and degenerative changes indifferent regions of rat brain in dose dependent manner. Kishandar (2007) reported neuro histopathological changes in rat brain treated with neonicotinoid insecticide imidaclorpid. Siraj Mohiyuddin et al.,(2009) reported neuro histopathological changes in rat brain regions treated with Acephate. Siva Prasad et al., (2014) observed the similar changes in Mice treated with Acephate. Devisrilakshmi Kala et al.,(2014) reported neuro histopathological changes in rat brain regions treated with Dimethoate.

Chlorpyrifos has been shown to disrupt the basic cellular machineries that control the patterns of neural cell maturation, and the formation and activity of synapses, exclusive of the effects on cholinesterase, which are mediated instead by its metabolite, chlorpyrifos oxon (Barone et al., 2000; Yanai et al., 2002; Gupta et al., 2004). Daily oral gavage and the associated repetitive stress are likely to exacerbate developmental toxicity and neurotoxicity, including that associated with organophosphate administration (Srilatha, 2012). Neurodegeneration was present in Cerebral cortex, dentate gyrus, and CA1 and CA3 subfields of the hippocampal formation and purkinje cells of the cerebellum. Neuronal degeneration of hippocampal cells is in consistent with OP compound induced alteration in behavior, and cognitive deficits such as
impaired learning and memory (Kassa et al., 2001). Shih et al., (2003) demonstrated that lethal doses of tested nerve agents (i.e., tabun, Sarin, Soman, Cyclosarin, VR and VS) induced seizures accompanied by neuropathological lesions in the brain of guinea pig, similar to those lesions reported for other OP’s in other species. (Clement and Broxup, 1993; Petras, 1994). A recent study has described the early neuropathological changes like severe tremors, seizures and convulsions accompanied by damage involving mainly the cerebral cortex, the hippocampal formation (dentate Gyrus, and CA1, and CA3 subfields) and the cerebellum in the adult male rat brain after 24 h of exposure to a single intramuscular dose of Sarin.

Sarah Greish et al., (2011) studied the risks involved in dietary intake of profenofos exposed vegetables and reported that Profenofos causes severe histopathological changes in liver and kidney of Albino rat. Manal et al., (2008) reported severe histopathological alterations in different animals exposed to Profenofos. They reported that Profenofos exposed animals showed severe congestion and hemorrhage in the meningeal and cerebral blood vessels with neuronal degeneration and deeyelination of nerve fibres. Nashwa et al., (2012) reported a significant increase in the percentage of sperm morphological abnormalities in profenofos exposed rats. Fatma et al., (2007) reported that Profenofos induced histopathological changes in liver, kidney, spleen and testis. The liver of Profenofos exposed animals showed hepatic cell damage with degenerative changes.

Abeer and Fayroz (2010) studied the repeated dose of Chlorpyrifos in male Albino rats found decreased body weight, decreased thyroid weight decrease in size of follicles and amount of colloid focal degeneration of follicle cells, thickened collagen fibers and congested blood vessels were observed.

From the present study it can be concluded that Profenofos induced neuroanatomic alterations. The neurohistopathological changes observed in the present investigation and the literature cited above clearly illustrate the neurotoxic potentiality of Profenofos. The overall results of this study has demonstrated that the oral administration of sublethal dose of Profenofos has lead to the cytoarchitectural damage of cells in different regions of rat brain in time and days dependent manner indicating that neurohistopathological evaluation in pesticide exposed animals play a crucial role in screening for potential neurotoxicants with risk for humans.
Fig. 5.1

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Fig. 5.13

Fig. 5.14
LEGEND FOR FIGURES

**Fig. 5.1:** Group I (Control) rat cerebral cortex showing the pyramidal cells (PC), Golgi cells (GOL.C), glial cells (GC) and Neurofibrillar network are present - H & E 400X.

**Fig. 5.2:** Group II rat cerebral cortex under Profenofos showing with loss of neuronal process (LNP), slight congestion of blood vessel (LCBV), pyramidal cells (PC) and mild (M. Hae) - H & E 400X.
LEGEND FOR FIGURES

**Fig. 5.3:** Group III rat cerebral cortex under Profenofos showing loss of neuronal process (LNP), mild congestion of blood vessel (MCBV), Mild hemorrhage (M. Hae) and vacuolation (V) - H & E 400X.

**Fig. 5.4:** Group IV rat cerebral cortex under Profenofos showing pyknosis of neurons (Pyc.N), moderate congestion of blood vessel (MCBV), virchow robins space (VRS) and vacuolation are present in outer pyramidal layer - H & E 400X.
LEGEND FOR FIGURES

Fig. 5.5: Group I (Control) rat Hippocampus showing the glial cells (GC), pyramidal cells (PC), neurons with neuronal processes (N) and neurofibrillar network (NFN) between the nerve cell bodies - H & E 400X.

Fig. 5.6: Group II rat Hippocampus under Profenofos showing vacuolation (V), glial cells (GC), neurons with neuronal processes (N) and loss of neurofibrillar network (LNFN) – H & E 400X.
LEGEND FOR FIGURES

**Fig. 5.7:** Group III rat Hippocampus under Profenofos showing vacuolation (V), dilated blood vessel (DIBV), pyramidal cells (PC), Loss of architectural details (LAD), slight necrosis (SN) and degenerated neuron (DN) - H & E 400X.

**Fig. 5.8:** Group IV rat Hippocampus under Profenofos showing moderate congestion of blood vessel (MCBV), congestion of glial cells (CGC), congestion blood vessel (CBV), dilated blood vessel (DIBV) and loss of architectural details (LAD) - H & E 400X.
LEGEND FOR FIGURES

**Fig. 5.9:** Group I (Control) rat Cerebellum showing the Neurofibrillar network (NFN), glial cell (GC) and with architectural details (AD) - H & E 400X.

**Fig. 5.10:** Group II rat Cerebellum under Profenofos showing vacuolation (V), glial cell (GC), neurons with neuronal processes (N) and Loss of neurofibrillar network (LNFN) –H & E 400X
LEGEND FOR FIGURES

**Fig. 5.11:** Group III rat Cerebellum under Profenofos showing vacuolation (V) in neuro fibrillar network, dilated blood vessel (DIBV), Loss of architectural details (LAD), slight necrosis (SN) and degenerated neuron (DN) - H & E 400X.

**Fig. 5.12:** Group IV rat Cerebellum under Profenofos showing moderate congestion of blood vessel (MCBV), congestion of glial cells (CGC), congestion blood vessel (CBV), dilated blood vessel (DIBV) and loss of architectural details (LAD) - H & E 400X.
**LEGEND FOR FIGURES**

**Fig. 5.13:** Group I (Control) rat Medulla oblongata showing the neurofibrillar network (NFN), glial cell (GC), pyramidal cells (PC) and Neuronal cell (NC) - H & E 400X.

**Fig. 5.14:** Group II rat Medulla oblongata under Profenofos showing clumping of neuronal cells (CL.NC), dilated blood vessel (DIBV), granular cell (GC), neuronal cell (NC), degenerated granular cell (DGC), degenerated neuronal cell (DNC), Vacuolation (V) and excessive vacuolation (EV) - H & E 400X.
LEGEND FOR FIGURES

**Fig. 5.15:** Group III rat Medulla oblongata under Profenofos showing moderate congestion of blood vessel (MCBV), vacuolation (V), pyknotic nucleus (PYN), loss of architectural details (LAD) and dilated blood vessel (DIBV) - H & E 400X.

**Fig. 5.16:** Group IV rat Medulla oblongata under Profenofos showing vacuolation (V), mild congestion blood vessel (MCBV), loss of neurofibrillar network (LNFN)– H & E 400X.
LEGEND FOR FIGURES

Fig. 5.17: Group I (Control) rat Thyroid gland showing the Follicles (F), Follicular lumen (FL), colloid (C), Follicular Epithilium (FE) - H & E 400X.

Fig. 5.18: Group II rat Thyroid gland under Profenofos showing Slight vacuolated colloid (SVC), Epithilium cells (EC) and C-Cell (C) - H & E 400X.
LEGEND FOR FIGURES

**Fig. 5.19:** Group III rat Thyroid gland under Profenofos showing Moderate differentiated Follicles (MDF), Slight hemorrhage Follicular lumen (SHFL), Disturbed Epithelial cells (DEC) - H & E 400X.

**Fig. 5.20:** Group IV rat Thyroid gland under Profenofos showing Poorly differentiated Follicles (PDF), Hemorrhage Follicular lumen (HFL) and Vacuolated colloid (VC) – H & E 400X.