

# Cytoarchitectural Changes in Cerebral Cortex Toxicated With Dimethoate in *ALBINO RAT*

Devisrilakshmikala.S<sup>1</sup>, Jacob doss.P<sup>2</sup>

Department of Zoology, S.V. University, Tirupati, A.P., India

**Abstract:** Dimethoate (DM) is an organophosphate insecticide, has been used worldwide in agriculture and domestic for several years which has led to variety of negative effects in non target species including humans. Therefore, the present study investigated the acute oral sub lethal dose of Dimethoate on Cytoarchitecture of cortex region of brain viz., Cerebral cortex. The LD<sub>50</sub> of dimethoate (330 mg/kg BW) was evaluated by probit analysis method (Finney, 1971). Albino rats were divided into 4 groups with group I serving as control, whereas II, III and IV groups were given (1/10<sup>th</sup> LD<sub>50</sub> i.e., 330 mg/kg bw) orally single, double and multiple doses of Dimethoate respectively. After stipulated time period the rats were sacrificed by cervical dislocation and the brain tissues were isolated at cold conditions. The neuro histopathological changes were observed through light microscope. The changes in different brain regions were in time and days dependent manner.

**Keywords:** Dimethoate, histopathology, Cerebral cortex, Albino rats

## 1. Introduction

The use of Pesticides causes severe environmental and health hazards to organisms. Organophosphate (OP) compounds are widely used and include some of the most toxic chemical agents. Due to their high insecticidal activity, low environmental persistence and moderate toxicity, the OP compounds are the most favoured insecticides. OP pesticides are known to cause inhibition of Acetyl cholinesterase (AChE) activity in the target tissues, which accumulates acetylcholine and prevents the smooth transmission of nerve functions leading to respiratory distress, convulsions, coma and death. Exposure to pesticides at any point in the life cycle has the potential for causing a range of short term or long term health problems. Exposure to pesticides at certain developmental stages of life can result in irreversible damage to organ structure and function.

Dimethoate preliminary acts on CNS as nerve poison. Though pathologic examination of the nervous system is an important component of neurotoxicology, the features of brain such as cellular complexity, regional variation in structure and function multiplicity that pesticides are responsible for many adverse effects fishes and other animals from the histopathological point of view.

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 in the study of microanatomy of specific tissues, has been successfully employed as a diagnostic tool in medical and veterinary sciences since the first cellular investigations carried out in the nineteenth 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, 2009).

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 into the tissue lesions prove to the external manifestations of the deleterious effects of heavy metals or any chemical.

Several studies have demonstrated that pesticides such as organochlorines, organophosphates, carbamates and pyrethroids induced embryo toxicity, genotoxicity, teratogenicity and tissue damage (Cavas and Ergene, 2003)

The aim of this paper is to illustrate the neuro histological changes in brain region viz., Cerebral cortex of Albino rat exposed to oral administration of sublethal doses of Dimethoate in time and days dependent manner.

## 2. Materials and Methods

### Pesticide

Dimethoate, technical grade of 97% purity was obtained from Hyderabad chemicals limited, Hyderabad, India.

### Animals

A total of 40 male Wistar strain rats (age 90-100 days, weight 200+ 10g) were used in the present study. They were housed five per cage and were maintained on a 12 h light/dark cycle in a temperature controlled (22<sup>o</sup> C) colony room and allowed to access to standard food and water *ad libitum*.

### Experimental design

The Albino rats were divided into four groups, with each group containing 10 rats. Of the 4 groups of rats, Group I served as control where the groups II, III and IV were given orally 10 days (1/10 LD<sub>50</sub> i.e., 330 mg/kg), 20 days and 30

days of Dimethoate respectively with 48 hrs of interval (i.e., on alternate days). The Group II on 11<sup>th</sup> day, Group III on 21<sup>th</sup> day, and Group IV on 31<sup>th</sup> day was sacrificed in a time and duration schedule ranging from 10 days to 30 days. The brain region such as cerebral cortex were isolated immediately at cold conditions.

### Neurohistopathology

At the end of experimental period treated rats were sacrificed. The isolated brain region were gently rinsed with physiological saline (0.9% NaCl) to remove blood debris and were fixed in 5% formalin for 24 hours. The fixative (formalin) was removed by washing with running tap water. After dehydrating through a grade series of alcohols, the tissue were clear in methyl benzoate and embedded in paraffin wax. Sections were cut at 6µ thickness and stained with Harris hematoxylin and counter stained with eosin dissolved in 95% alcohol. After dehydration and clearing, section were mounted in Canada balsam. Then the slides were observed under light microscope.

## 3. Results

Histopathological studies of brain region to evaluated the extent of neurotoxicity of dimethoate in time and days dependent manner. The damage to normal histology of cells was observed in Cerebral cortex region of brain studied. Changes in cortex region of brain were in accordance with the time and days dependent manner. Severe changes were observed in group IV and lowest in group II was with moderate neuropathology changes.

### 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.

- 1<sup>st</sup> layer – Consists of mostly glial cells, axons of neurons of other layers and very few neurons.
- 2<sup>nd</sup> layer – Small pyramidal cells
- 3<sup>rd</sup> layer - Large pyramidal cells
- 4<sup>th</sup> layer – Rich with stellate and granule cells which receive input to the cortex from thalamocortical fibers, association fibers and commissural fibers.
- 5<sup>th</sup> layer – Largest pyramidal cells known as giant pyramidal cells or Betz cells
- 6<sup>th</sup> layer – Martinotti cells.

Microphotograph of control rat Cerebral cortex shows different layers such as molecular layer with glial cells, pyramidal layer with large and small pyramidal cells and neurofibrillar network (Plate 5.1 and Fig.A).

### Histology of rat Cerebral cortex under Dimethoate toxicity

10 days treated rat cortex showed vacuolation (V), glial cells (GC), pyramidal cells (PC) and dilated blood vessel (Plate 5.1 and Fig.B).

After 20 of days administration, the cortex showed the dilated blood vessel (DIBV), mild hemorrhage (MH), degenerated neurons (DN) and vacuolation (Plate 5.2 and Fig.C).

In 30 days treated rat cortex, the cytoarchitectural changes were remarkably more distinct, which include mild hemorrhage (M.Hae), loss of neuronal processes (LNP), loss of architectural details (LAD), pyramidal cells (PC) and vacuolation (V) were observed (Plate 5.2 and Fig.D).

### Legend for figures

#### Cerebral Cortex

**Fig. A:** Control rat cerebral cortex showing the pyramidal cells (PC), glial cells (GC) and Neurofibrillar network are present – H & E 400X.

**Fig. B:** Group I Rat cerebral cortex under Dimethoate showing vacuolation and dilated blood vessel – H & E 400X.

**Fig. C :** Group II Rat cerebral cortex under Dimethoate showing the dilated blood vessel (DIBV), mild haemorrhage, degenerated neurons and vacuolation - H & E 400X.

**Fig. D:** Group III rat cerebral cortex under Dimethoate showing mild haemorrhage, loss of neuronal processes (LNP) loss of architectural details (LAD) and vacuolation are present in outer pyramidal layer – H & E 400 X

## 4. Discussion

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 artefacts to occur in nervous tissue samples unless scrupulous attention is paid to dissection, sampling fixation, processing, sectioning and staining of the material.

The present study has clearly revealed the cytoarchitectural changes in cortex region of rat brain administered with 10 days, 20 days and 30 days of Dimethoate. The neuro histopathological changes were more pronounced in 30 days treated rats, than those of 20 days and 10 days treated rats. The observations were in agreement with OP induced neurotoxicity. Histopathological parameters were studied to evaluate the extent of neurotoxicity of Dimethoate in time and days dependent manner. The damage to normal cytoarchitecture of cells was observed in almost cortex region of brain studied. Severities of changes in different regions of brain are in accordance with the time and days regimen. Severity was highest in 30 days treated rats and least in 10 days treated rats with the 20 days treated rats showing intermediary neuropathologic changes. The changes in cell dynamics of Dimethoate treated rat cortex showed mild hemorrhage, loss of neuronal processes, loss of architectural details, Pyramidal cells and vacuolation.

Calson *et al.*, (2000) observed OP compound 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 (Miles *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.

Mukhopadhyay *et al.*, (2004) reported cypermethrin caused DNA damage in brain ganglia of *drosophila melanogaster*. 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.

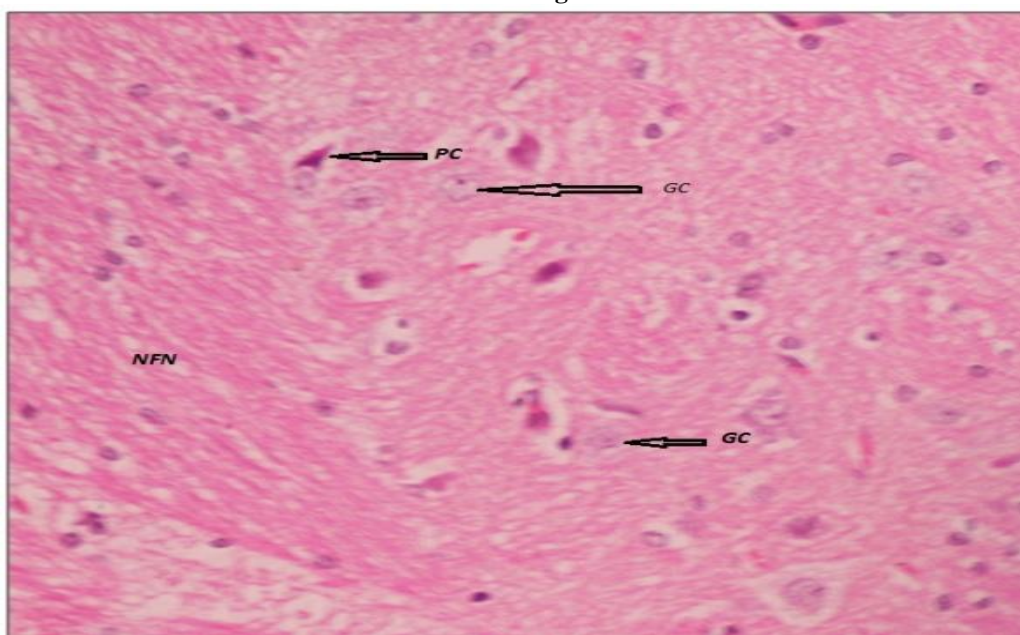
Earlier Latuszynski *et al.*, (1999) reported that the dermal exposure of chlorpyrifos and cypermethrin leads to the several histopathological changes as well as increased Cerebral cortex 5.1

density of the cytoplasm in focal pyknosis of the cytoplasm in the cerebral cortex, cerebri and the cerebellum.

Intoxication by OP compound soman causes prolonged seizures that lead to neuro pathology in the brain (Bhagat *et al.*, 2001). A recent study has described the early neuropathological changes in the adult male rat brain 24 hrs after exposure to a single intramuscular dose of 1.0, 0.5, 0.1 or 0.01 X LD<sub>50</sub> (100 µg /kg) Sarin. Sarin at 1.0 X LD<sub>50</sub> Caused severe tremors, seizures and convulsions accompanied by damage involving mainly the cerebral cortex, the hippocampal formation (dentate Gyrus, and CA<sub>1</sub>, and CA<sub>3</sub> subfields) and the cerebellum.

From the present study it can be concluded that Dimethoate induced neuroanatomic alterations. The neuro histopathological observations and the literature cited above clearly illustrated or summarizes the neurotoxic potentiality of Dimethoate. The overall results of this study has demonstrated that the oral administration of sublethal dose of OP compound Dimethoate has lead to the cytoarchitectural damage of cells in Cerebral cortex region of rat brain in time and days dependent manner.

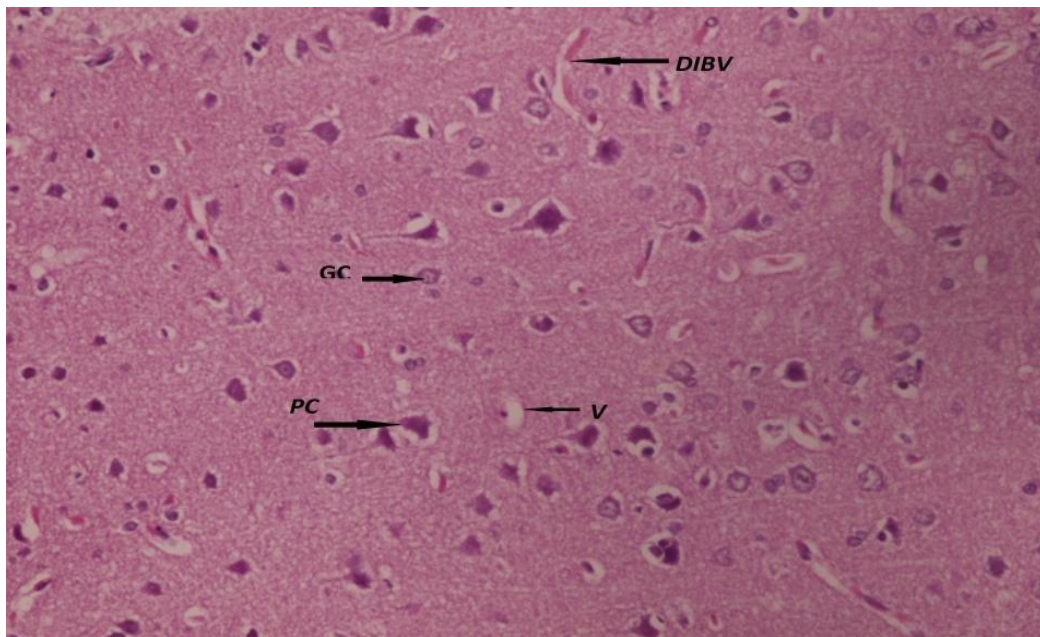
Fig.A



**Figure A:** Control rat cerebral cortex showing the pyramidal cells (PC), glial cells (GC) and neurofibrillar network are present – H & E 400X.

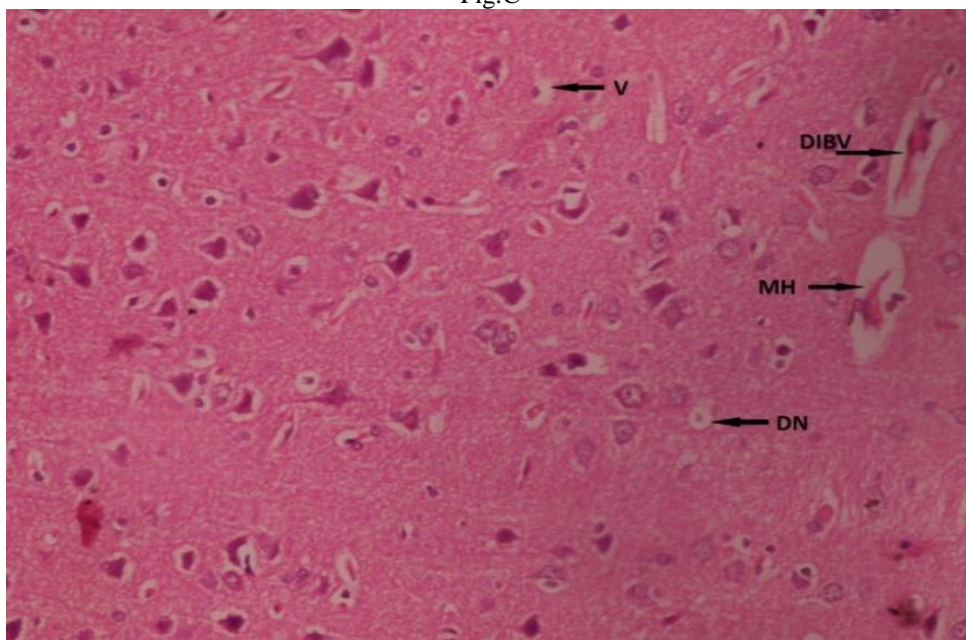
Fig.B





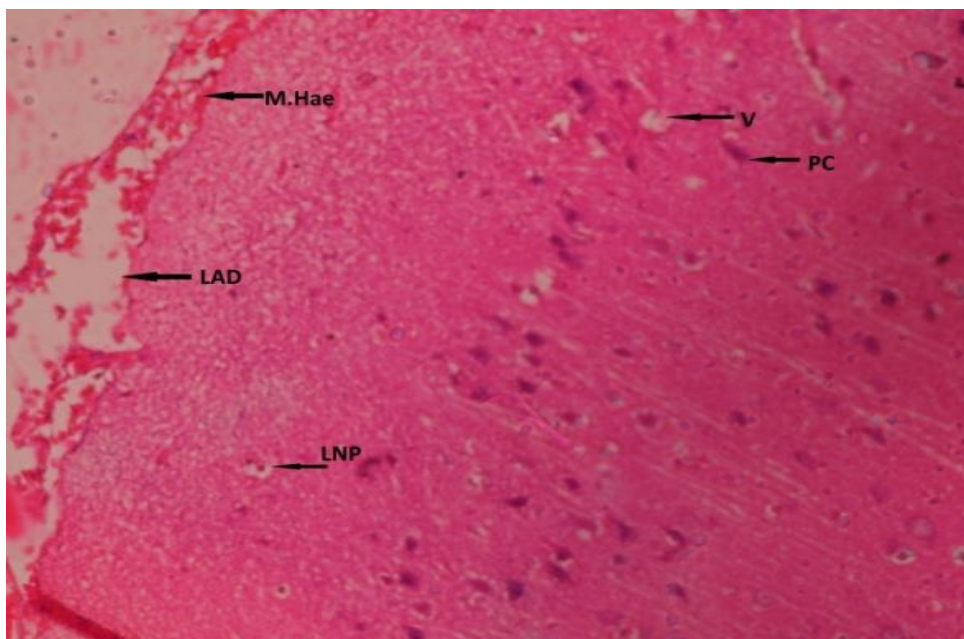
**Figure B:** 10 days rat cerebral cortex under Dimethoate showing vacuolation (V), glial cells (GC), pyramidal cells (PC) and dilated blood vessel— H & E 400X.

Cerebral cortex 5.2  
 Fig.C



**Figure C :** 20 days rat cerebral cortex under Dimethoate showing the dilated blood vessel (DIBV), mild hemorrhage (MH), degenerated neurons (DN) and vacuolation (V) - H & E 400X.

Fig.D



**Figure D:** 30 days rat cerebral cortex under Dimethoate showing mild hemorrhage (M.Hae), loss of neuronal processes (LNP) loss of architectural details (LAD), pyramidal cells (PC) and vacuolation (V) – H& E 400 X

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