Histopathological Studies of Neonicotinoid Insecticide Imidacloprid on Different Regions of Albino Rat Brain

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Abstract

Imidacloprid is a neonicotinoid insecticide being used extensively for crop protection and pet flea control programmes. Present study was aimed to investigate the toxic effect of imidacloprid on histopathological studies in different regions of brain of albino rats. Rats were treated orally with imidacloprid as single, double and multiple doses with intervals of 48 hrs and up to 25th day of 1/5th LD90 i.e. 80mg/kg bw was given. Total proteins showed decrement, whereas free amino acids, the activities of protease, aspartate amino transferase, alanine amino tansferase and glutamate dehydrogenase are significantly increased in imidacloprid exposed rats. Thus variation in the protein metabolic profiles of the rat exposed to imidacloprid indicates its toxic effect on the cellular metabolism there by leading to impaired protein synthetic mechanism is clearly evidenced by histopathological studies. The histopathological changes were observed by hematoxylin and eosin staining.

Key words: Imidacloprid, histopathology, different regions of brain, Albino rat.

1. Introduction

The discovery of neonicotinoids as important novel pesticides can be considered a milestone in insecticide research of the past three decades. Neonicotinoids represent the fastest-growing class of insecticides introduced to the market since the commercialization of pyrethroids (Nauen and Bretschneider, 2002). Like the naturally occurring nicotine, all neonicotinoids act on the insect central nervous system (CNS) as agonists of the postsynaptic nicotinic acetylcholine receptors (nAChRs) (Bai et al., 1991; Liu and Casida, 1993a; Yamamoto, 1996; Chao et al., 1997; Zhang et al., 2000; Nauen et al., 2001). Although previous studies have found low toxicity to mammals (M.Antra-Cordone and P. Durkin, 2005) and humans, human imidacloprid poisoning (D.David et al., 2007). As a result of this mode of action there is no cross-resistance to conventional insecticide classes, and therefore the neonicotinoids have begun replacing pyrethroids, chlorinated hydrocarbons, organophosphates, carbamates, and several other classes of compounds as insecticides to control insect pests on major crops (Denholm et al., 2002). Today the classes of neonicotinoids are part of a single mode of action group as defined by the Insecticide Resistance Action Committee (IRAC; an Expert Committee of Crop Life) for pest management purposes (Nauen et al., 2001).

Histopathology, the microscopic study of diseased tissue, is an important tool of anatomical pathology. In terrestrial and aquatic animals, insecticides produce toxic effects on different tissues. For understanding the pathological conditions of the animal, histological studies pave a way, to have a clear understanding as to how these insecticides cause injury to the tissues. It is essential to have an insight in to the histological analysis of the tissues. Some pesticides are toxic at even very low concentrations and these pesticides necessarily impairs the metabolic strategy of the animal physiologically and structurally. Pesticide that enter the body via internal digestive system after oral administration. They are not subjected initially either to the detoxifying reactions of the liver or to excrete via the urinary system. Compounds transported by oral feeding in effect can be distributed to all parts of the body in their unmetabolised form (Turner and Shanks, 1980). Moreover, immunological, biochemical and neurobehavioural deficits were found in rats exposed to imidacloprid (M. B. Abou-Donia et al., 2008; S.Bhardwaj et al., 2010; V. Duzguner and S. Erdogan, 2010; M. Mohany et al., 2011.) Susceptibility to chemical injury varies greatly among the tissue and cell of the same animal and more so among
different animal groups. The extent of severity of tissue damage in a function of the concentration and potentiality of toxic compound accumulated in the tissues as it is time dependent (Jayantha Rao, 1982). Histopathological changes with different pesticides have been reported earlier. (Frick et al., 1971; Newmaun and Maclean, 1974; Couch, 1977; Peguiguot et al., 1978; Kumar and Pant, 1981 and Usha Rani, 1986). But the literature on histopathology of imidacloprid on rat is scanty. Hence an attention has been made in the present investigation. In the present study, the main concentration on different regions of rat brain i.e., cortex, hippocampus, cerebellum and medulla have been taken. All these regions are critically involved in the coordination and maintenance of the body. There are detailed studies of developmental immunotoxicity of imidacloprid in wister rats (Lalita Gawade et al., 2013)

2. MATERIALS AND METHODS

2.1. Test chemical: Imidacloprid technical (92%) was obtained from Bayer crop science limited, Mumbai.

2.2. Animal model: Albino rat

Wistar strain white rats were selected as experimental animals for the present study. Rats of 5-6 months, weighing 200±30 grams were taken as a group. Animals were housed four per cage with free access for food and water ad libitum. The animals were maintained in the animal house at 25±1°C with a day night cycle of 12 hours. The food provided to animals was standard laboratory feed (Hindustan Lever Ltd., Mumbai). All the hygienic practices were followed during the maintenance of animals.

2.3. Experimental Design:

Healthy adult rats were divided into four groups having twelve animals each. Toxicity of imidacloprid was evaluated by static bioassay method of Finney and the LD50 of imidacloprid to albino rat was found to be as 400mg/kg bw. 1/5 of LD50 value of imidacloprid (i.e 80mg/kg bw) was selected as sub lethal dose. The fist group animals treated as vehicle control animals. To the second group of animals, single dose of imidacloprid (i.e. on 1st day) was administered orally. Double doses were given with 48h interval to the third group of animals on 1st and 3rd day. To the fourth group of animals, multiple doses were given with 48h interval i.e., on 1st, 3rd, 5th, 7th and so on up to 25th day through oral gavage. After 48h, both control and experimental animals were sacrificed and brain regions isolated and stored in -80oC for histopathological studies.

2.4. Histopathology:

Histopathological examination of the tissues was followed as per Humason (1972). Brain tissues like cerebellum, hippocampus, medulla and cerebral cortex were isolated from the control and experimental animals. They were gently rinsed with physiological saline solution (0.9% NaCl) to remove blood and debris adhering to the tissues. They were fixed in 5% formalin for 24 hours. The fixative was removed by washing through running tap water for overnight. After dehydration through a graded series of alcohol, the tissues were cleared in methyl benzoate, embedded in paraffin wax. Sections were cut of 6 µ thickness and stained with hematoxylin and eosin (H&E). After dehydration and clearing, the sections were mounted in DPX mount and observed under microscope.

3. Results

3.1. Rat cerebral cortex under imidacloprid

In control the cerebral cortex consisting of molecular layer (ML), outer granular layer (OGL) and outer pyramidal layer (OPL). In outer pyramidal layer nerve cell bodies and pyramidal cells (PC) are present. In higher magnification outer pyramidal layer neuroglia cells (NGC), betz cells (BC) and nerve cell bodies (NCB) are present. Cerebral cortex of rat under multiple dose of imidacloprid represents four layers with degeneration in betz cells (DGBC), horizontal cells (DGHC) and necrosis in neural cell body (NNCB). Necrosis also occurs in nerve cell bodies (Fig. 1.a, 1.b).

3.2. Rat hippocampus under imidacloprid

Control hippocampus of rat consisting of two layers of Cornu Ammonis (CA1 and CA3). In between CA1 and CA3 glial cells and nerve cell bodies are present. A thick fibrillate network in neuropil (NP) is present. In higher magnification different sizes of glial cells (GC) and nerve cell bodies (NCB) are present in between CA1 and CA3 layers. Hippocampus of rat under multiple dose of imidacloprid. Under multiple dose administration vesicular nucleus contained glial cells (VNGC), less number of nerve cell bodies and glial cells glial cell bodies are totally degenerated and appears as a vesicular nucleus (Fig. 2.a, 2.b).

3.3. Rat cerebellum under imidacloprid

In control cerebellum consisting of Molecular layer (ML) with an appearance of a tree. Within the granular layer a layer of purkinje cells (PJC) were noticed. The Cerebellum of rat under multiple dose of imidacloprid administration showing clear necrotic changes in purkinje cells (NPJC) in granular layer. The photomicrograph shows degenerative
changes in purkinje cells (DGPC) of granular layer, cellular degenerative changes (CDGML) and severe necrotic changes (SNML) in molecular layer (Fig 3.a, 3.b).

**CEREBELLM**

**3.4. Rat medulla under imidacloprid**

Microphotographs show control medulla consisting of neuropil (NP), nerve cell bodies (NCB) and glial cells (GC) with an appearance of fibrillary back ground. In multiple dose administration of imidacloprid, clear necrosis in gray matter neuropil and severe necrosis in white matter neuropil is observed. Soft puffy and edemaition condition, numerous cystic spaces were occurred in gray matter and white matter. Degeneration occur in nerve cell bodies and glial cells. In higher magnification severe necrosis in neuropil (SNNP), degeneration in nerve cell bodies (DGNPB), condensation in nerve cell bodies (CNCB) and degeneration in glial cells (DGCC) (Fig. 4.a, 4.b).

**MEDULLA**

X400 Magnifications

**4. DISCUSSION**

The present studies are related to histopathological changes on different regions of brain on imidacloprid and the first time these studies are being reported. It is clearly indicated that imidacloprid induced marked pathological changes in different brain regions of rat administered with multiple doses. Severe pathological lesions were observed in multiple doses rather than single and double dose. In the present study various pathological changes observed in different brain regions of experimental rat, were condensation of nuclear material in neurons, degenerative changes in glial cells, neuropil, congestion in blood vessels and vesicular bodies. Necrosis occurs in gray matter and white matter.

Nervous tissue has presented extraordinary challenges to science. Historically, a basic appreciation of the cellular composition of nervous tissue did not come until decades after other tissues were fairly well understood. Neurodegeneration was observed in cerebellum, hippocampal area CA1, when administered double and multiple dose of imidacloprid. Neurodegeneration was evident in cerebellum, hippocampal area CA1, and hypothalamus. Neuronal loss could be observed in parietal cortex, dedifferentiation was found in hypothalamus and stratum and monoaminergic, cholinergic, and amino acidergic deficits were shown in several brain regions (Guenther Bernert et al., 2003). Neurodegeneration with neuronal death, glial proliferation and neurotransmitter changes has been shown in mouse administered with methamphetamine (Schmued and Bowyer 1997).

During the normal development of the cerebral cortex the granule cell proliferation and migration to the inner granular layer and deeper parts of the molecular layer are complete by the end of the postnatal days. Many researchers have interpreted that a narrow molecular layer may result due to the reduce dendritic arborization at the purkinje cells (MC Connell and Beny, 1978; Gopinath, 1984).

It has also been clearly observed that vascular damage is focal and neuronal tissue in the vicinity of such damage presents patchy degeneration. Kousten and Norton (1991) explained such changes and their resultant as a reduction in blood supply to the nervous tissue which results in several types of alteration including generalized hypertension and capillary damage. It is also believed that primary damage to capillaries is an acute response to high levels of cytotoxic agents such as heavy metals like arsenic (Harding et al., 1968) and lead (Pounds, 1984).

Histologically the evolution of brain specimens from rats exposed to imidacloprid in different doses dissolved the presence of different histopathological changes in rats were, necrosis in pyramidal cells, betz cells, pyknosis of cytoplasm in nerve cell bodies. Previously Latuszynska et al., (2001; 2003) reported some histopathological changes in various areas of the brain as well as increased density of the cytoplasm in neurocytes as a result of dermal application of chloropyrifos and cypermethrin in rats. The changes observed were most is striking in the cells of (Cornu Ammonis) CA1 and CA3 hippocampus layers, the hypothalamus and the stratum granulosum in area dentate. As reported by others focal pyknosis of the cytoplasm was observed in the cortex cerebri and the cerebellum. Latuszynska et al., (2001, 2003; Sivaiah, 2006). Luty et al., (1998) also reported that changes concerning the purkinje cells in the cerebellum, concentration of the cytoplasm of single pyramidal cells of CA3 hippocampus layer as a focal pyknosis at the neurocytes at nuclei lateralis hypothalamai and the cerebral cortex in rats exposed to 250 mg / kg alpha cypermethrin.

Recently there are many histopathological studies took place in different parts of rat like liver, heart, kidneys etc. The results are in accordance with histopathological lesions observed in liver of male rats (M.Mohany et al., 2011), female rats (Harmandeep Kaur Toor et al, 2013), Japanese quail exposed to imidacloprid in layer chickens (A.M. Kammon et al, 2010).Intoxication by the organophosphate compound causes prolonged seizures that lead to neuropathology in the brain (Bhagat et al., 2001,....
Rajendra Prasad 2007, Sukanya 2008, Siraj Mohiyouddin 2008). In conclusion the overall results of this study clearly demonstrate that oral administration of the imidacloprid leads histopathological changes in the brain regions. The neonicotinoid has also been observed to damage the developing vasculature in cerebellum. Harmandeep kaur Toor, et al., (2013) also reported that Imidacloprid induced histological and biochemical alterations in liver also found in female albino rats. Chronic exposure to imidacloprid also induces inflammation and oxidative stress in the liver and central nervous system of rats (V. Duzguner, S. Erdogan, 2012).

5. References:

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