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Research Article

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Histopathological and ultrastructural changes induced by imidacloprid in brain and protective role of vitamin C in rats

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ABSTRACT

In the present study, the effect of oral administration of imidacloprid over 4 weeks on histological and ultrastructural alterations in brain was assessed in rats. Forty eight male rats were divided into four groups of 12 animals each. Group 1 is control, group 2 was administered with imidacloprid at the rate of 80 mg/kg b.wt/day, group 3 was fed with vitamin C at the rate of 10 mg/kg b.wt/day and group 4 was treated with both imidacloprid and vitamin C for 4 weeks. In group 2, brain section revealed marked congestion in cerebellum, degeneration of purkinje cells with loss of dendrites, vacuolation around neurons and shrunken neurons, vacuolation around neuronal cell body and chromatolysis. The sections of group 4 revealed mild congestion and degeneration of purkinje cells on whereas group 3 animals did not reveal any significant pathological changes. Ultra thin sections of brain revealed vacuolar mitochondria, apoptotic nuclei with disrupted and margination of chromatin material. Group 4 brain section revealed degeneration of neurons. In conclusion, these results suggest that exposure to imidacloprid (80 mg/kg) in male rats induced histological and ultrastructural alterations brain, and co-administration of vitamin C brought moderate protection.

Keywords*:* imidacloprid, brain, vitamin C

INTRODUCTION

Indiscriminate usage of pesticides in agriculture is leading to contamination of environment and natural resources, and thereby producing an adverse impact on animal and human health. Imidacloprid is a neonicotinoid insecticide and classified under toxicity class II /III agents by United States Environmental Protection Agency [10]. It is extensively used against various sucking insects *viz.*, aphids, leaf hoppers, thrips, white fleas, leaf miners, beetles [4] and also used as foliar treatment for soil and for seed dressing [1]. In Veterinary Medicine, it is used as flea control agent on dogs and cats [5] and also used to control houseflies on poultry farms. It is one of the fastest sold insecticide across the world because of its high selective toxicity in insects and apparent safety in humans [7]. Its selective toxicity results from its high affinity to insect's nicotinic acetylcholine receptors compared to mammals [6]. It acts on nervous system by blocking post-synaptic acetylcholine receptors, which kills the insect [7]. A case of acute poisoning was reported in human following ingestion of a pesticide formulation containing 10% imidacloprid [12] and two fatal intoxication cases have been reported recently [8]. Although it is widely used in worldwide, there

is still less work done related to its toxicity in male rats. Therefore, a study has been designed to evaluate the histological and ultra structural alterations in brain induced by imidacloprid in male rats.

EXPERIMENTAL SECTION

Chemicals

Imidacloprid was procured from GSP crop science Pvt. Ltd., Gujarat and vitamin C was obtained from Abbott Health Care Pvt. Ltd., Bhiwandi.

Experimental Design

Forty eight male *Sprague dawley* rats, weighing 200-250 g, were procured from National Institute of Nutrition (NIN), Hyderabad, India. The experiment was conducted as per CPCSEA guidelines and prior approval by the Institutional Animal Ethics Committee (Approval No. I / 3 / 2012). The rats were housed in solid bottom polypropylene cages at lab animal house in the Department of Pharmacology & Toxicology, College of Veterinary Science, Hyderabad and were maintained in controlled environment (Temperature 20-22^{0}C) throughout the course of the experiment. Rice husk was used as bedding material. All the rats were provided *ad libitum* with standard pellet diet (procured from NIN) and water throughout the experimental period. Following an acclimatization period of one week, the animals were divided into four groups consisting of 12 in each. Group 1 served as control, group 2 was treated with imidacloprid at the rate of 80 mg/kg b.wt, group 3 was treated with vitamin C at the rate of 10 mg/kg b.wt and group 4 was treated with both imidacloprid and vitamin C. These drugs were administered by oral gavage every day consequently for 28 days. At the end of 14th day six rats from each group, remaining at the end of 28th day were sacrificed by cervical dislocation.

Histopathological examination

Respective tissue samples from brain were collected and fixed in 10% neutral buffered formalin. After washing in running water and dehydration in alcohol, tissues were embedded in paraffin and 5µm sections were cut and stained with Hematoxylin and Eosin as per the method of Luna [3].

Ultrastructure pathological examination

Soon after sacrifice thin slices of brain tissues were dissected into 2.5% gluteraldehyde in 0.1M phosphate buffer (pH 7.3 stored at 4^{0}C), washed in buffer, post fixed in 1% osmium tetraoxide in 0.1M phosphate buffer, dehydrated with ascending grades of acetone, embedded in Spurr's resin and were incubated over night at 60^{0}C for complete polymerization of the tissue. Semi thin (1000 -1500 nm thickness) sections were made with ultra microtome, stained with 1% toludine blue to locate exact area to be sectioned for TEM. Then, ultra thin sections were made (500 -700 nm thickness) mounted on hexagonal copper grids, allowed to air dry for over night and were stained with saturated urenyl acetate and 1% Reynolds's lead citrate as per the protocol of [2]. All grids were dried at room temperature and observed under transmission electron microscope.

RESULTS AND DISCUSSION

In group 2, brain sections revealed marked congestion in cerebellum (Fig. 1), degeneration of purkinje cells with loss of dendrites (Fig. 2), vacuolation around neurons and shrunken neurons (Fig. 3) on 14th day of experiment. On day 28, brain sections revealed vacuolation around neuronal cell body, chromatolysis (Fig. 4) and marked congestion (Fig. 5). The sections of group 4 revealed mild congestion (Fig. 6) and mild degeneration of purkinje cells (Fig.7) on 28th day of experiment, whereas group 3 animals did not reveal any significant pathological changes. These findings are in agreement with the earlier report [9].

Ultra thin sections of brain in group 2 revealed vacuolar mitochondria (Fig. 8), apoptotic nuclei with disrupted and margination of chromatin material (Fig. 9) on day 28. In group 4, brain section revealed degeneration of neurons (Fig. 10) on 28th day of experiment.

The changes recorded in this study might be due to accumulation of imidacloprid and its metabolites in the brain. Vitamin C plays a pivotal role in neutralizing free radicals; it can work both inside and outside the cells to combat free radical damage. The free radicals will seek out an electron to regain their stability, vitamin C is an excellent source of electrons so it can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench

their reactivity [11]. In the present study, supplementation of vitamin C brought moderate protection due to its antioxidant and free radical scavenging effect, resulted in repair and regeneration of damaged tissues

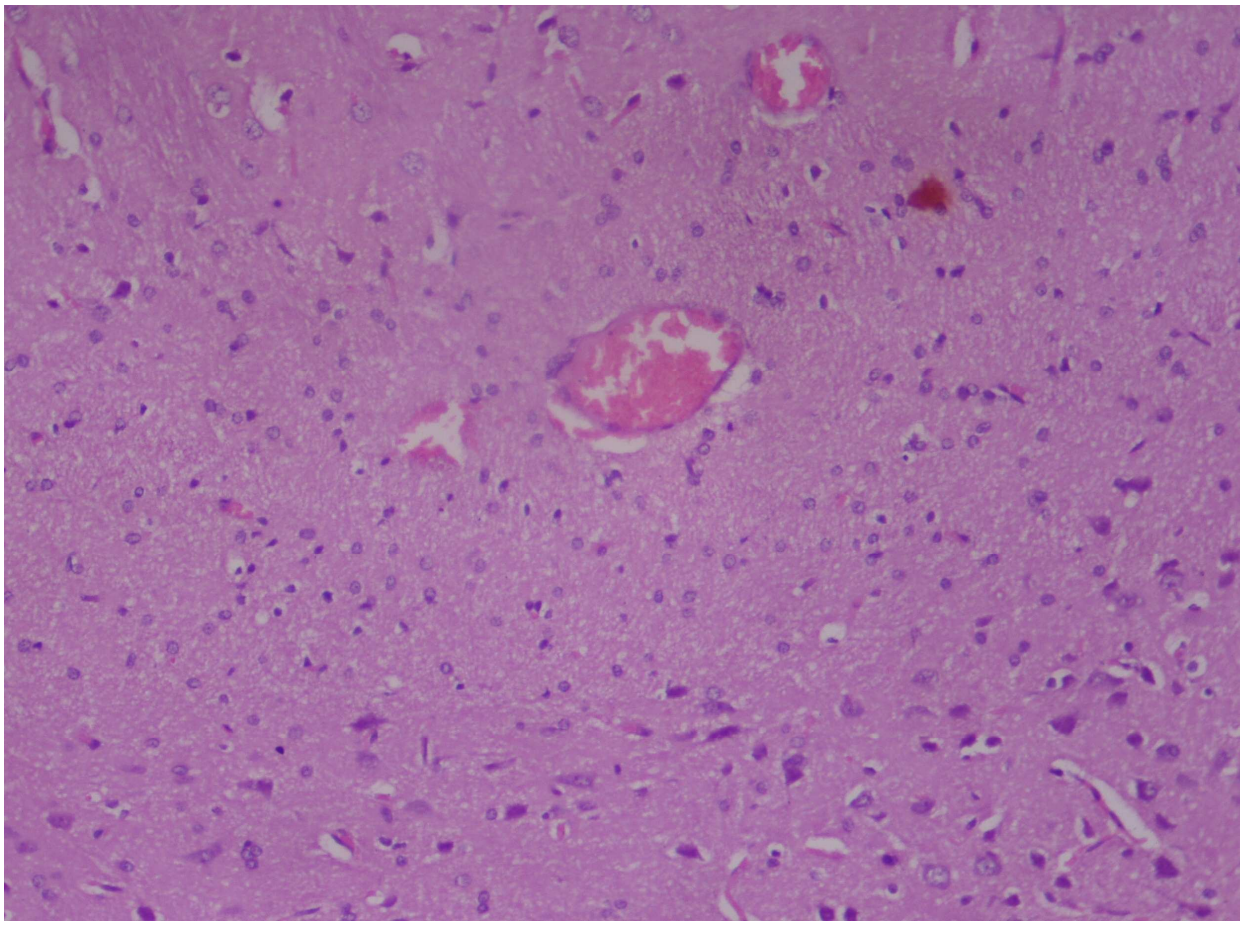

Fig. 1: Photomicrograph of brain showing marked congestion in cerebellum (Group 2, day 14): H&E X 200

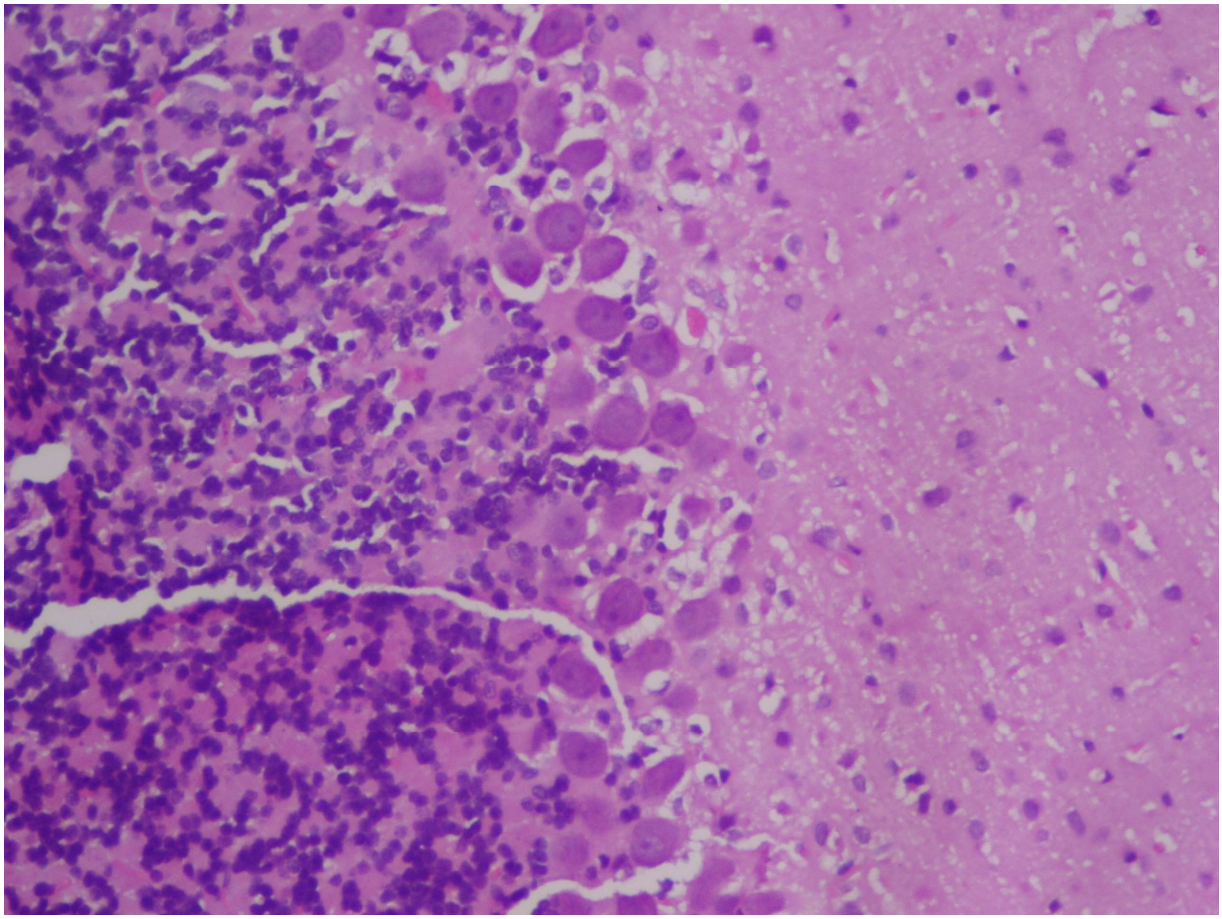

Fig. 2: Photomicrograph of brain showing degenerated purkinje cells with loss of dendrites (Group 2, day 14): H&E X 200

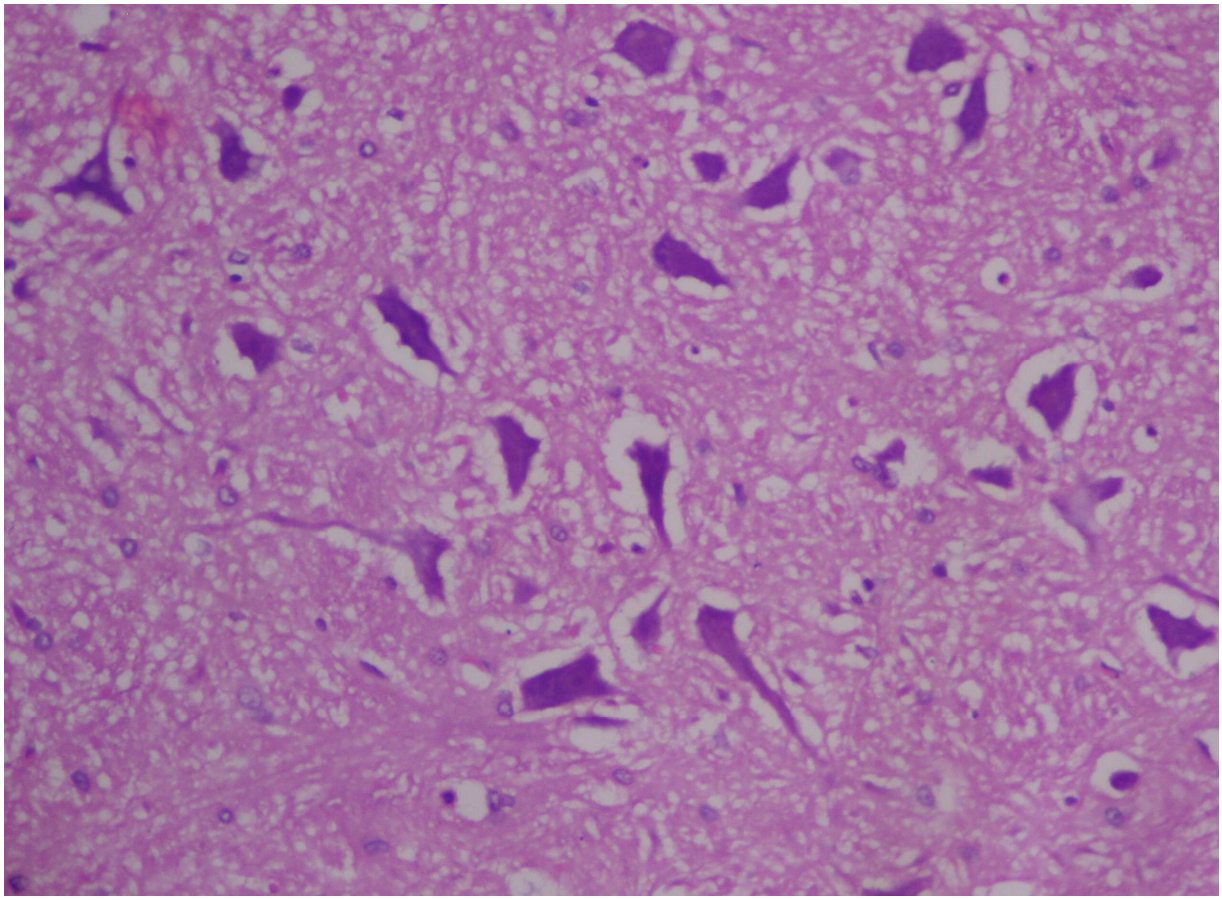

Fig. 3: Photomicrograph of brain showing vacuolation around neurons and shrunken neurons (Group 2, day 14): H&E X 200

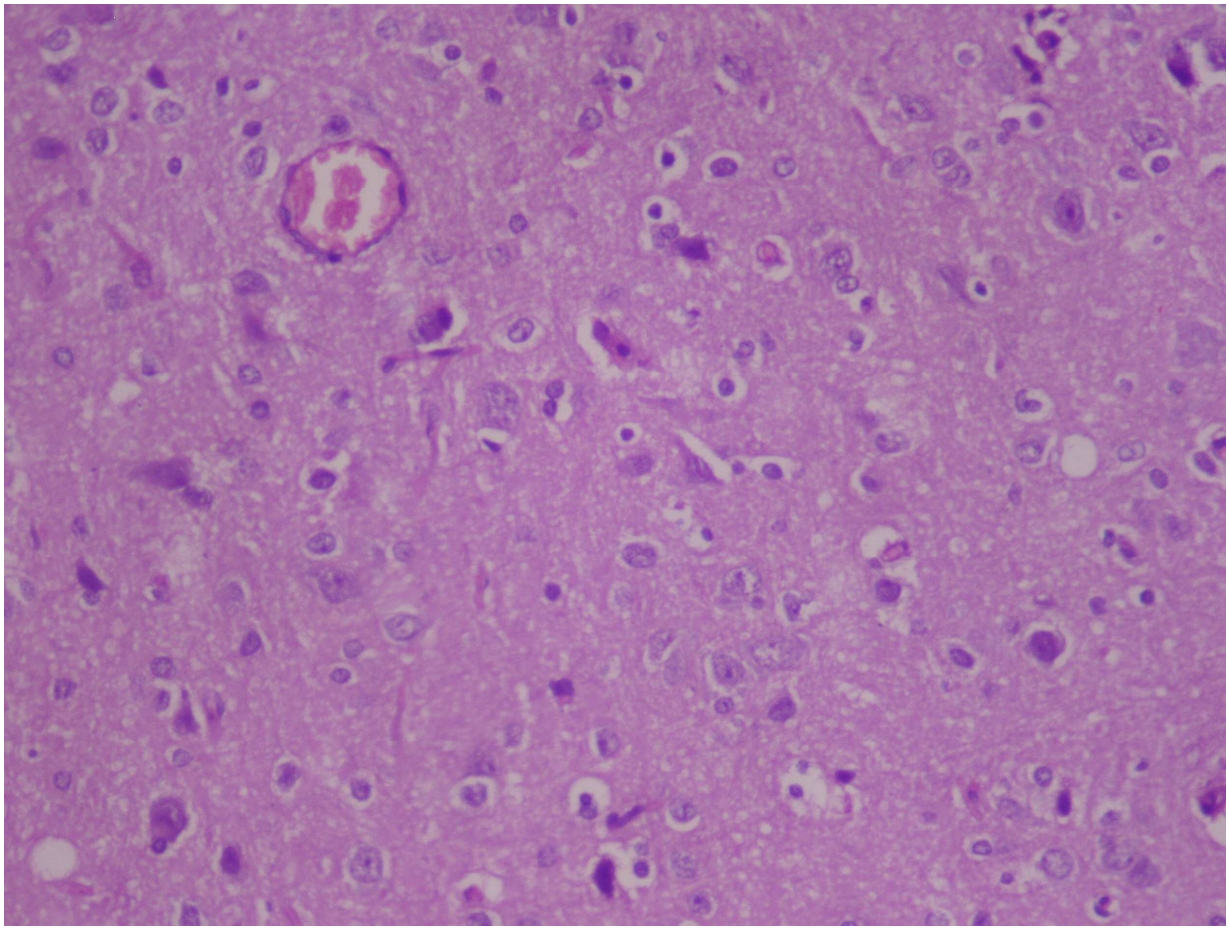

Fig. 4: Photomicrograph of brain showing congestion, vacuolation and chromatolysis in cerebellum (Group 2, day 28): H&E X 200

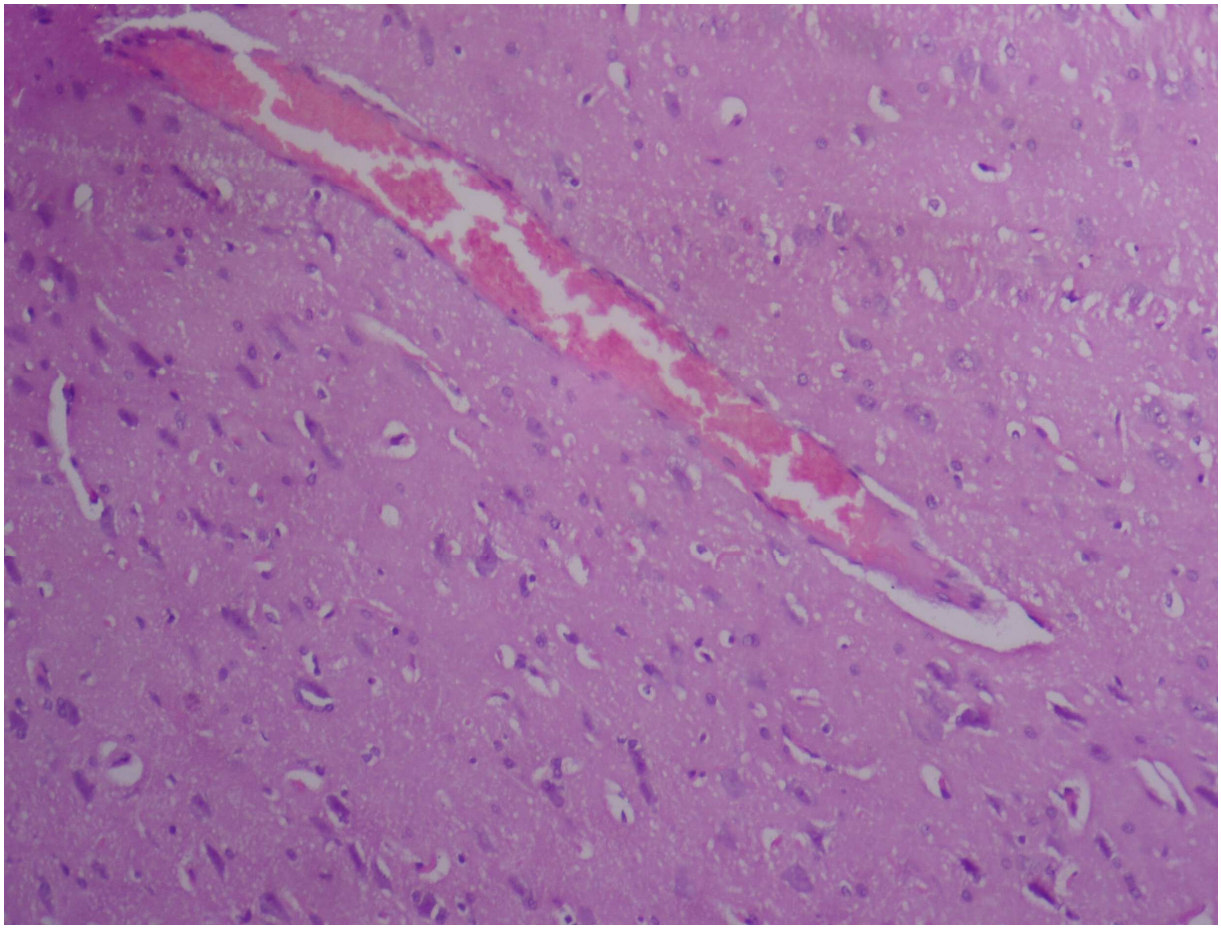

Fig 5: Photomicrograph of brain showing marked congestion in cerebellum (Group 2, day 28): H&E X 200

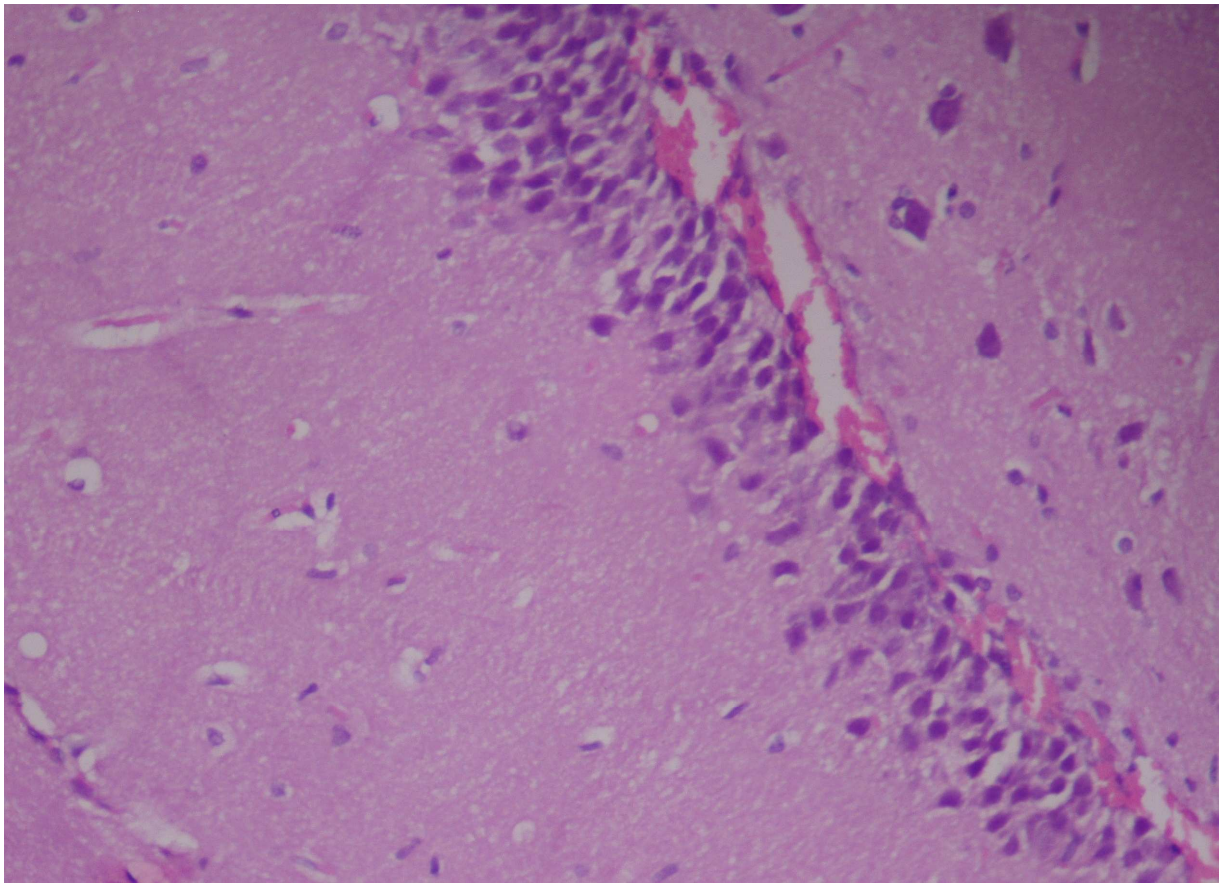

Fig. 6: Photomicrograph of brain showing mild congestion (Group 4, day 28): H&E X 200

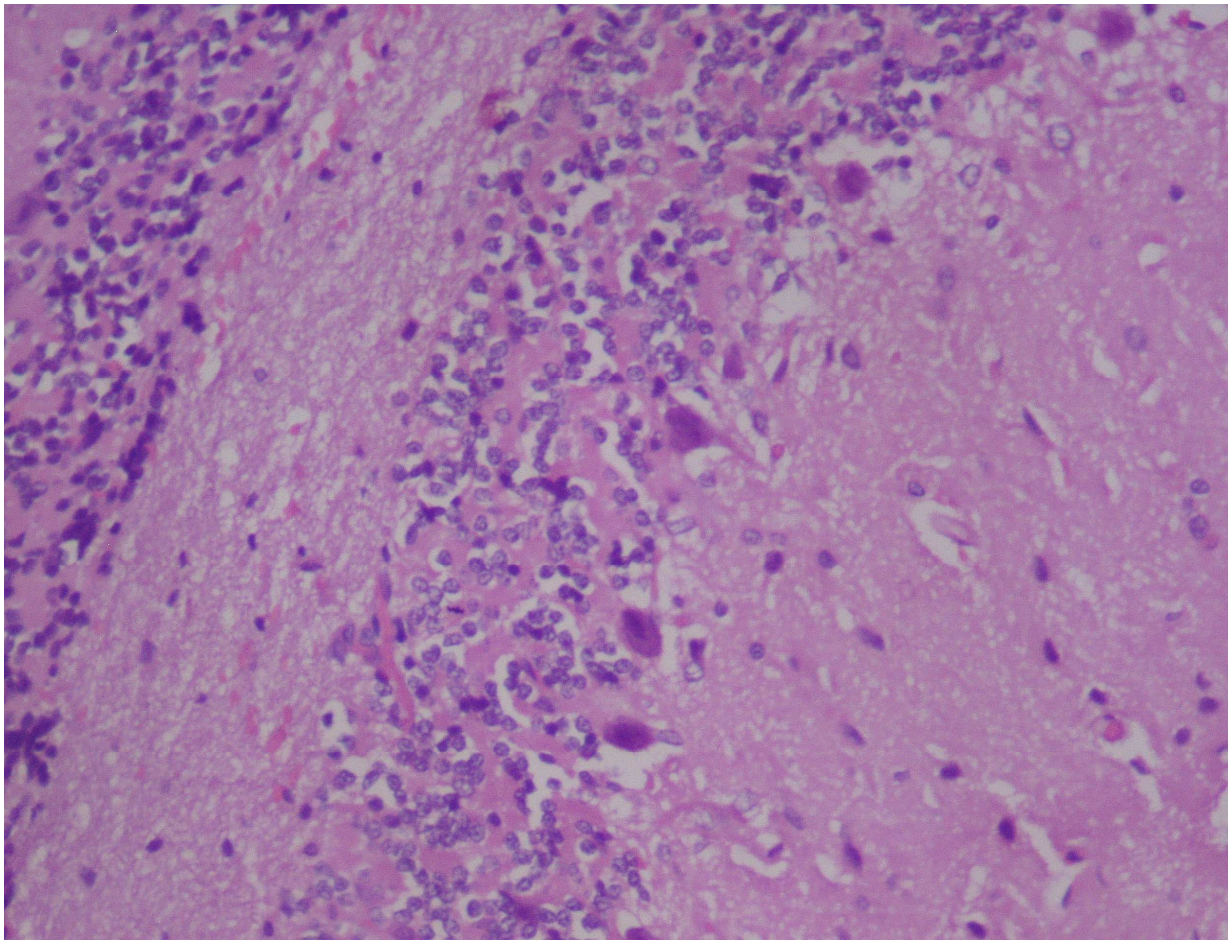

Fig. 7: Photomicrograph of brain showing mild degenerative changes in purkinje cells (Group 4, day 28): H&E X 200

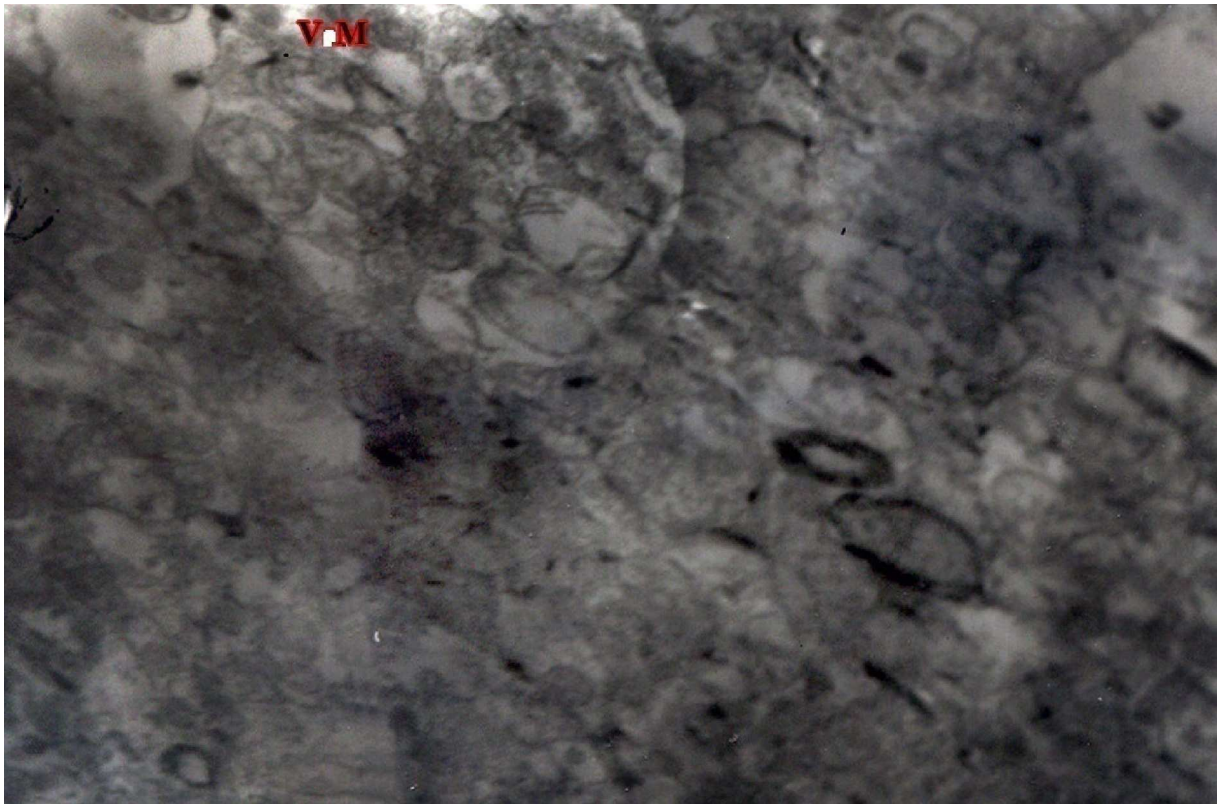

Fig. 8: TEM of brain showing vacuolar mitochondria (VM): UA & LC 9300 X (Group 2, day 28)

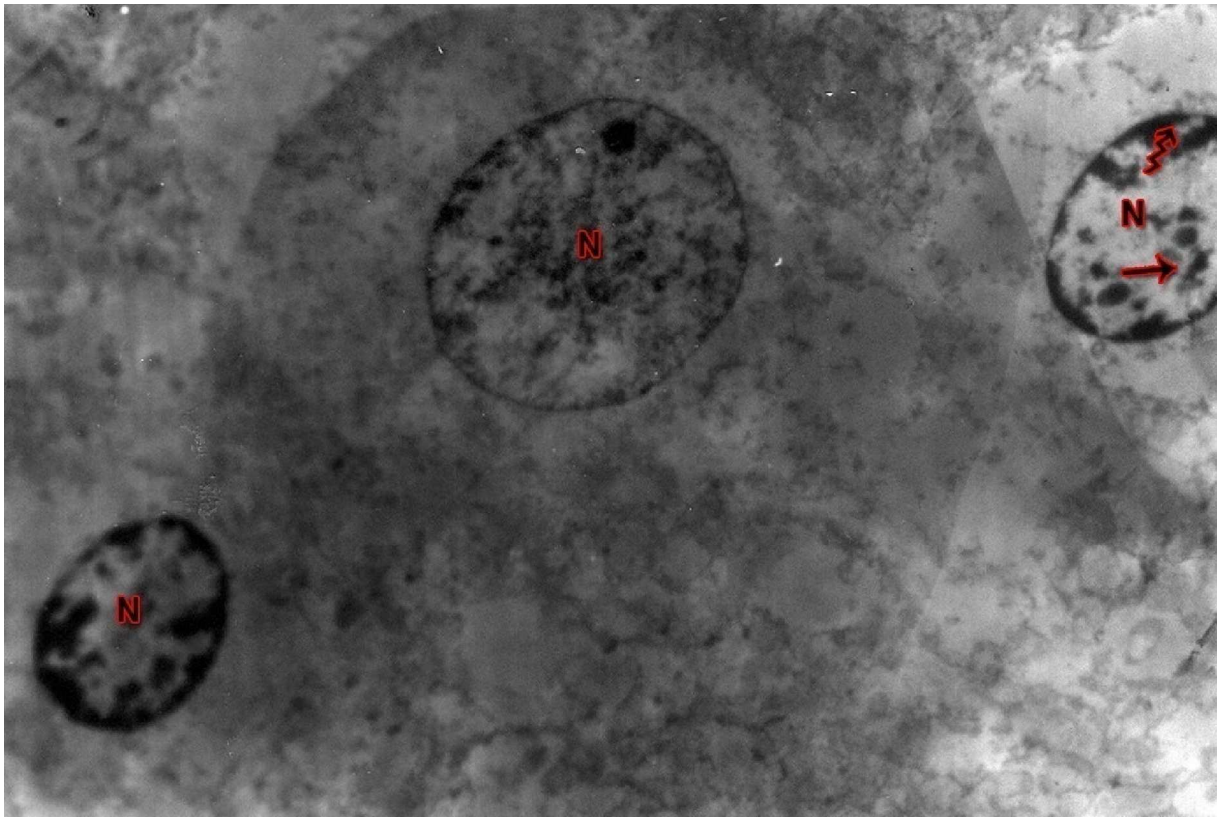

Fig. 9: TEM of brain showing apoptotic nucleus (N), disrupted and marginated chromatin material (arrow): UA & LC 4650 X (Group 2, day 28)

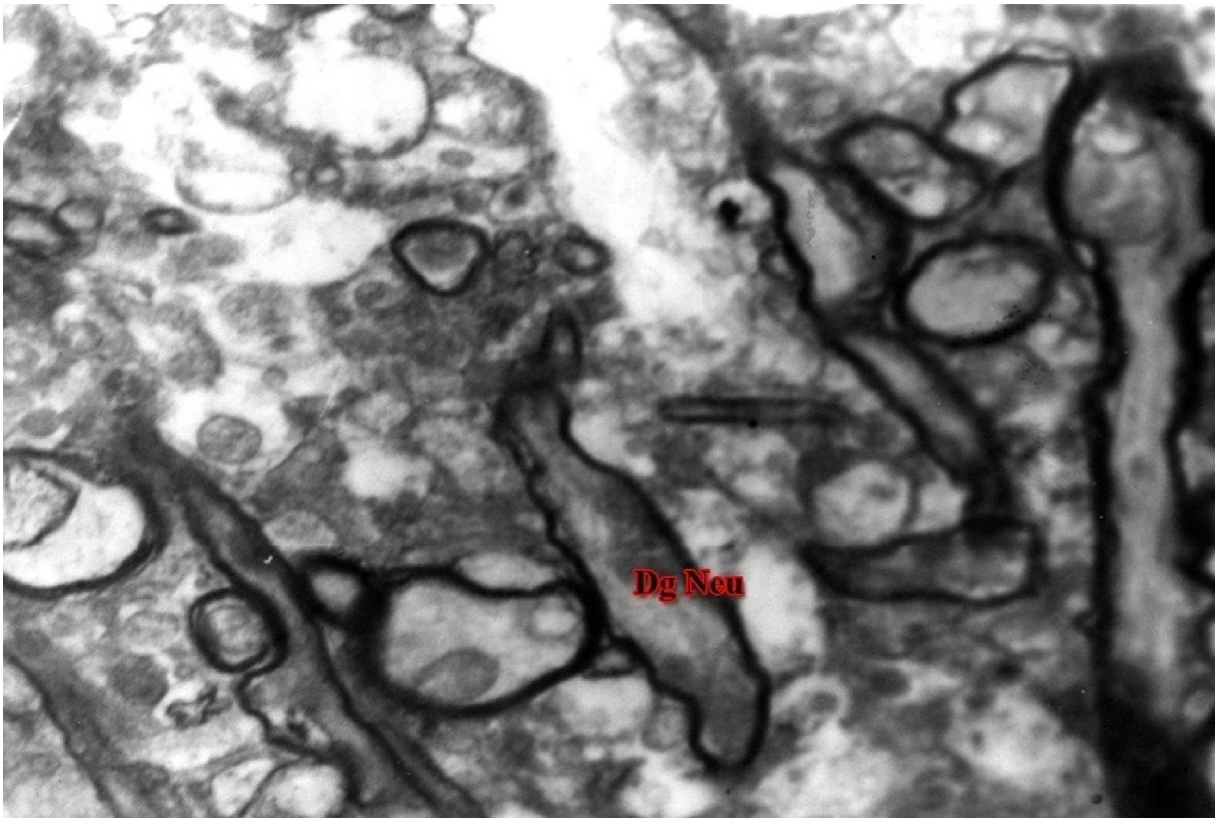

Fig. 10: TEM of brain showing degenerated neurons (DgNeu): UA & LC 7440 X (Group 4, day 28)

In conclusion, the study revealed that exposure to imidacloprid (80 mg/kg) resulted in neuro toxicity in rats, which was evident from histological and ultrastructural alterations in brain. However, vitamin C supplementation along with imidacloprid to rats, manifested significant protective effects.

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