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HISTOPATHOLOGICAL CHANGES IN CENTRAL NERVOUS SYSTEM OF RATS FOLLOWING ADMINISTRATION OF FLUORIDE

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ABSTRACT
Fluoride has the ability to interfere with the functions of the brain that causes impairment of central nervous system. In the present study young, healthy Wistar albino rats weighing 100-200 g were allocated to four groups. Group I was given 1 ml double distilled deionized water/kg body weight/day for forty days and kept as control. The remaining groups II, III and IV were treated with 100, 200 and 300 ppm of sodium fluoride/kg body weight/day via oral gavage for the same period. The rats were sacrificed and hematoxylin and eosin stained sections of cerebellum were studied for neuropathological alterations. In rat treated with 100 ppm sodium fluoride, the cerebellar cortex exhibited decrease in the number of Purkinje neurons. Some Purkinje neurons exhibited degeneration, distortion in the form of rounded, crescent and spindle shaped cell formation. Pyknosis of granule cells and atrophied nuclei of Purkinje neurons were seen in Purkinje neuron layer. In rat treated with 200 ppm sodium fluoride, neurofibrillary tangles in the granule layer and sign degenerative spaces around constricted Purkinje neurons were visible. Neuronophagia, pyknotic Purkinje neurons and loss of Nissl substance from neuroplasm were observed. The clumps of granular cells and degeneration in between granular cell layer were present. In cerebellar cortex of rat treated with 300 ppm sodium fluoride, the Purkinje neurons showed dot like nuclei. In the central medulla of white matter and granular layer, there were present fluid filled degenerating spaces with debris of dead granular cells and nerve fibers. In some Purkinje neurons, darkly stained, spindle formed nuclei were shifted to the periphery. Degenerative spaces in the Purkinje neuron layer, chromatolysis in the granule cells and perivascular edema around constricted blood capillaries were clearly observed. The neuropathological observations in cerebellar cortex of this study clearly reveal that the fluoride may be playing important role as causative agent for the neuronal cell damage and disruption of cerebellar integrity.

KEYWORDS: Cerebellum, Cerebellar cortex, Neuropathology, Purkinje Neuron, Sodium fluoride, Wistar albino rat.

INTRODUCTION
The most wide spread disorder due to increasing fluoride pollution in environment is fluorosis and impairment of soft tissue function. The earliest toxicity of fluoride affects several organs including brain in human and rats. Although the blood brain barrier is impermeable to fluoride, it did not pose an absolute barrier and fluoride had the ability to enter the brain as it crosses the blood brain barrier. Fluoride affects the structure and function of the brain (Shashi *et al*., 2008). Fluoride accumulates in the neurons and neuroglia showed morphological changes mainly in the hippocampus (Mullenix *et al*., 1995) and causes several pathological changes in the neurons (Ge *et al*., 2005; Shashi and Sharma, 2015). The cerebellum plays an important role in movement and posture. Malformation and lesions of the cerebellum disrupt motor coordination and impair balance. So in the light of earlier reported result, the present study was undertaken to investigate the neuropathological toxic effects of fluoride on cerebellum of Wistar albino rat.

MATERIALS AND METHODS
Albino rats weighing 100-200 g of Wistar strain were housed in polypropylene cages with stainless steel grill tops and bedded with paddy husk. They were provided free access of standard rat pellet diet and drinking water was provided *ad labitum*. The experiment was performed under the approved of Institutional Animal Ethics Committee, Punjabi University, Patiala (Approval no. 107/99/ CPCSEA -2012-10).

Study design
The animals were acclimatized to the laboratory conditions for two weeks and then they were divided randomly into four groups. Administration of sodium

fluoride via oral gavage lasted for forty consecutive days which was done as follows.

Group I: The animals of this group served as control and were given 1 ml double distilled water/kg body weight/day.

Group II: The animals of this group received sodium fluoride at a dose of 100 ppm/kg bw/day. *Group III*: The animals of this group received sodium fluoride at a dose of 200 ppm/kg bw/day. *Group IV*: The animals of this group received sodium fluoride at a dose of 300 ppm/kg bw/day.

The control and experimental animals were sacrificed under ether anaesthesia after 40 days of fluoride treatment. Brain was dissected out, cerebellum was separated and washed in normal saline and processed for neuropathological examination.

Neuropathological Examination

The cerebellum was fixed in 10% formalin, dehydrated in 95% alcohol for 45 minutes, tertiary-butyl alcohol for 6 hours, cleared in amyl acetate for overnight, and were embedded in Paraffin wax. Wax blocks were prepared and 7 µm thin serial sections were cut with rotary microtome and stained with hematoxylin and eosin. Sections were examined under microscope (Leica DM 2000) and subsequently photomicrographs were taken with camera (Leica DFC 450 C) fitted on research microscope.

RESULTS

The cerebellar cortex of control rat showed normal histology of three cortical layers. It showed well organized molecular layer (ML), Purkinje neuron layer (PNL), granular layer (GL), and the central medulla of white matter (WM) (Fig. 1). The Purkinje neuron layer was sandwiched between molecular and granular layer (Fig. 2). There were present normal pyriform shaped Purkinje neuron body with dendrites and centrally placed nucleus. There were visible intact granule cells with rounded heterochromatic nucleus (Fig. 3)

In rat treated with 100 ppm sodium fluoride, the cerebellar cortex showed decrease in the number of Purkinje cells and presence of empty spaces in the Purkinje neuron layer (Fig. 4). Some Purkinje neurons exhibited degeneration (Fig. 5). There were distortion in shape of Purkinje neuron in the form of rounded, crescent and spindle shaped cell formation (Fig. 6). Pyknosis of granule cells and atrophied nucleus of Purkinje neuron were seen (Fig. 7).

In rat treated with 200 ppm sodium fluoride, Purkinje cells were the most affected cells which were constricted structure leaving empty spaces around it. The neurofibrillary tangles were seen in the granular layer (Fig. 8). Destruction of neuron in the form of neuronophagia and pyknosis was exhibited by Purkinje cells along with loss of Nissl substance from neuroplasm (Fig. 9). There was accumulation of granule cells in the form of clumps leaving empty spaces in between granular cell layer (Fig. 10). The hyperatrophied nucleus occupied maximum available space in the Purkinje neuron (Fig. 11). Size of Purkinje cell was reduced and there was no differentiation of nucleus, neuroplasm and cell membrane as compared to control (Fig. 12). The fluid filled and vacuolated areas were visible in molecular layer which were result of degeneration of nerve fiber (Fig. 13).

In cerebellar cortex of rat treated with 300 ppm sodium fluoride, the Purkinje cells had dot like nucleus. The fluid filled degenerating spaces were visible in the central medulla of white matter and granular layer (Fig. 14). Debris of dead granular cells and nerve fibers were clearly visible in the granular cell layer (Fig. 15). In the Purkinje neuron, darkly stained, spindle shaped nucleus was shifted to the periphery (Fig. 16). Degenerating areas were observed in the Purkinje layer (Fig. 17). There was chromatolysis in the granule cells and perivascular edema around constricted blood capillaries (Fig. 18).

Explanation to figures

Figure 1. T. S of cerebellum of control rat showing three cortical layers molecular layer (ML), Purkinje neuron layer (PNL), granular layer (GL), and the central medulla of white matter (WM). H&E, X200.

Figure 2. T. S of cerebellum of control rat showing alignment of Purkinje neuron layer between molecular and granular layer, H&E X400.

Figure 3. T. S of cerebellum of control rat showing normal pyriform shaped Purkinje neuron perikaryon with dendrites and centrally placed nucleus (black arrow). The granule cells contain rounded heterochromatic nucleus (red arrow). H&E, X1000.

Figure 4. T. S of cerebellum of rat treated with 100 ppm sodium fluoride showing decrease in the number of Purkinje cells and presence of empty spaces in the Purkinje neuron layer, H&E X200.

Figure 5. T. S of cerebellum of rat treated with 100 ppm sodium fluoride showing degenerating Purkinje neuron in the Purkinje neuron layer. H&E, X400.

Figure 6. T. S of cerebellum of rat treated with 100 ppm sodium fluoride showing distortion in the shape of Purkinje neuron in the form of rounded, crescent and spindle shaped cell formation in the Purkinje neuron layer. H&E, X400.

Figure 7. T. S of cerebellum of rat treated with 100 ppm sodium fluoride showing pyknosis of granule cells and atrophy of nucleus in Purkinje neuron. H&E, X1000.

Figure 8. T. S of cerebellum of rat treated with 200 ppm sodium fluoride showing neurofibrillary tangles in the granule layer and empty space around constricted Purkinje cell. H&E, X200.

Figure 9. T. S of cerebellum of rat treated with 200 ppm sodium fluoride showing neuronophagia in the form of pyknotic Purkinje cells with loss of Nissl substance from neuroplasm. H&E, X400.

Figure 10. T. S of cerebellum of rat treated with 200 ppm sodium fluoride showing accumulation of granular cells in the form of clumps (black arrow) with increase in empty spaces (red arrow) in between granular cell layer. H&E, X400.

Figure 11. T. S of cerebellum of rat treated with 200 ppm sodium fluoride showing hypertrophied nucleus in the Purkinje cell. H&E, X1000.

Figure 12. T. S of cerebellum of rat treated with 200 ppm sodium fluoride showing reduced size with unclear differentiation of neuron boundary, neuroplasm and nucleus of Purkinje cell. H&E, X1000.

Figure 13. T. S of cerebellum of rat treated with 200 ppm sodium fluoride showing degenerative areas in the molecular layer. H&E, X1000.

Figure 14. T. S of cerebellum of rat treated with 300 ppm sodium fluoride showing Purkinje cell with dot like nucleus(red arrow) and fluid filed degenerating spaces in the central medulla of white matter and granular layer (black arrow). H&E, X400.

Figure 15. T. S of cerebellum of rat treated with 300 ppm sodium fluoride showing debris of dead granular cells and nerve fibers in the granular cell layer. H&E, X400.

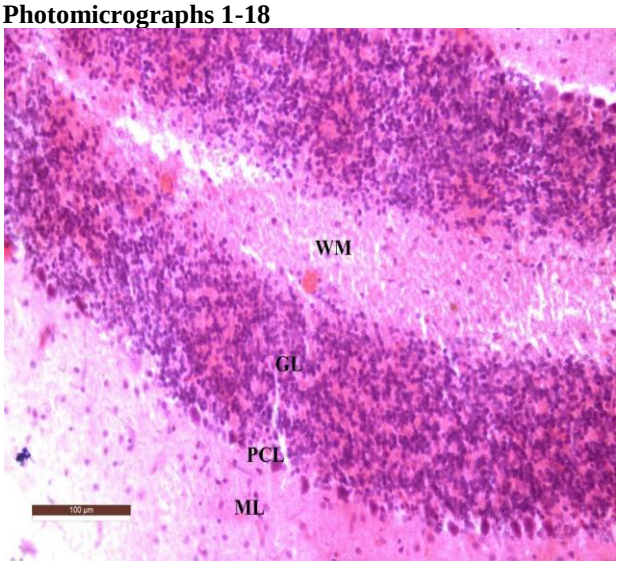
Figure 16. T. S of cerebellum of rat treated with 300 ppm sodium fluoride showing shifting of darkly stained, spindle formed nucleus to the periphery in Purkinje cell.

H&E, X1000.

Figure 17. T. S of cerebellum of rat treated with 300 ppm sodium fluoride showing decrease in the density in the Purkinje cell layer. H&E, X1000.

Figure 18. T. S of cerebellum of rat treated with 300 ppm sodium fluoride showing chromatolysis in the granule cells and perivascular edema around constricted blood capillaries. H&E, X1000.

Photomicrographs 1-18



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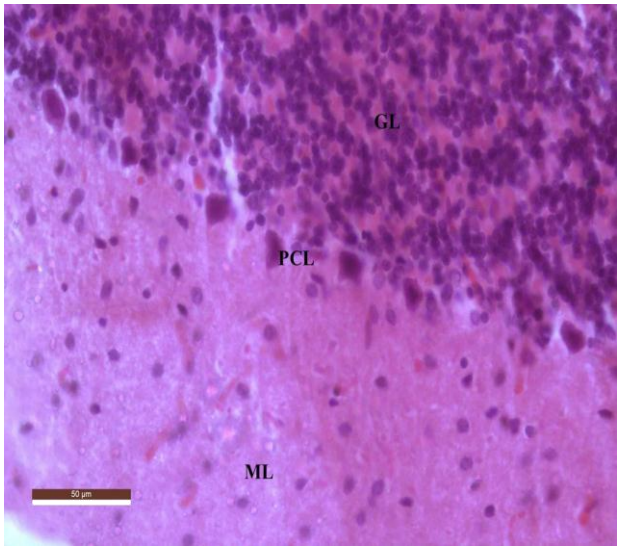

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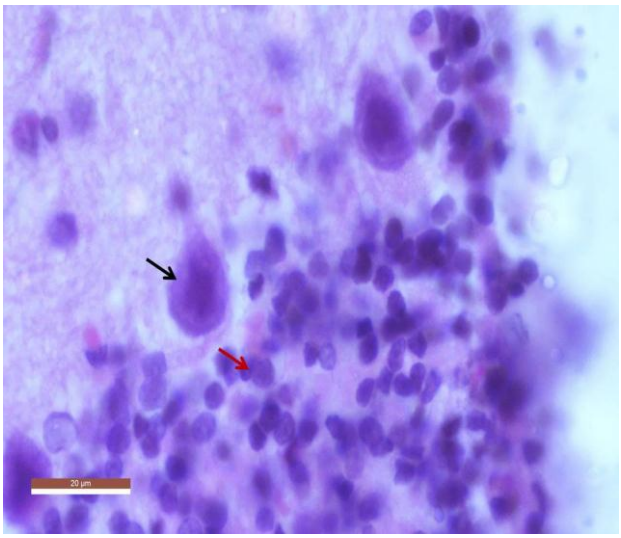

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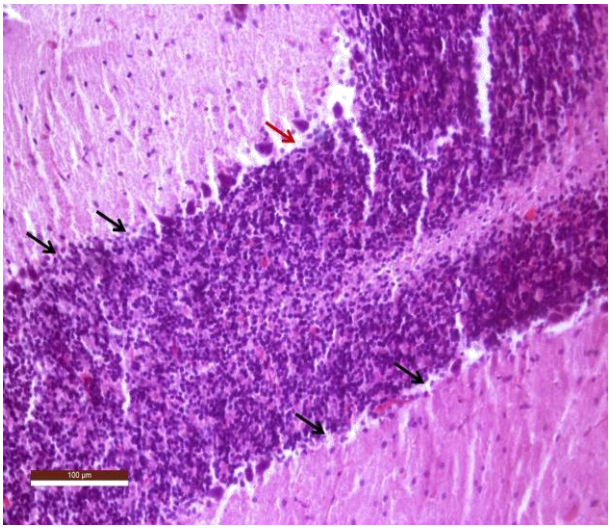

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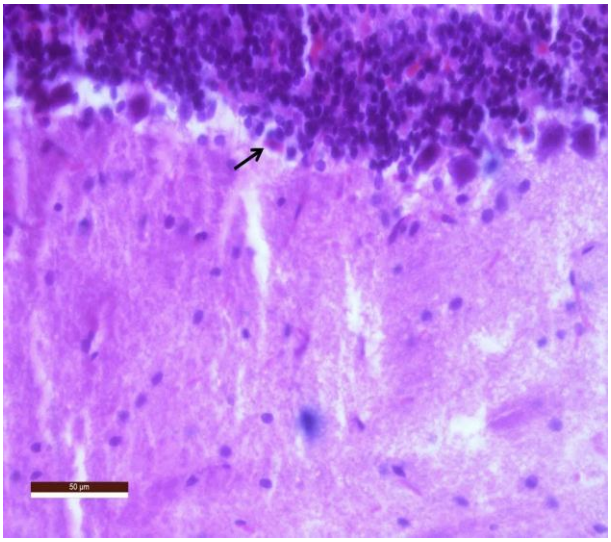

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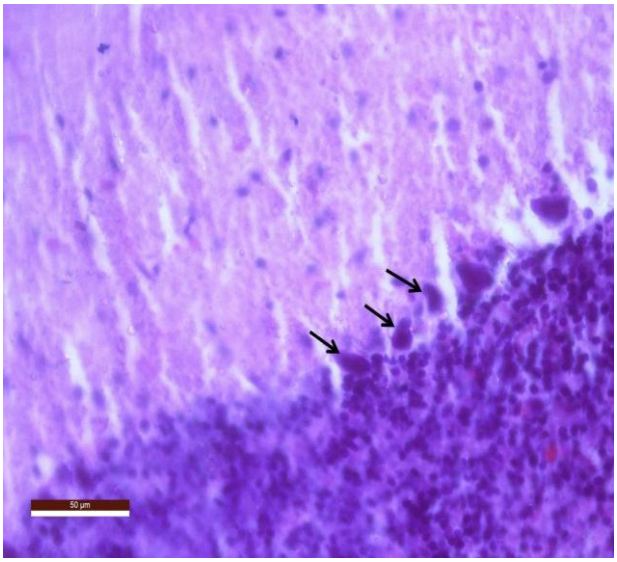

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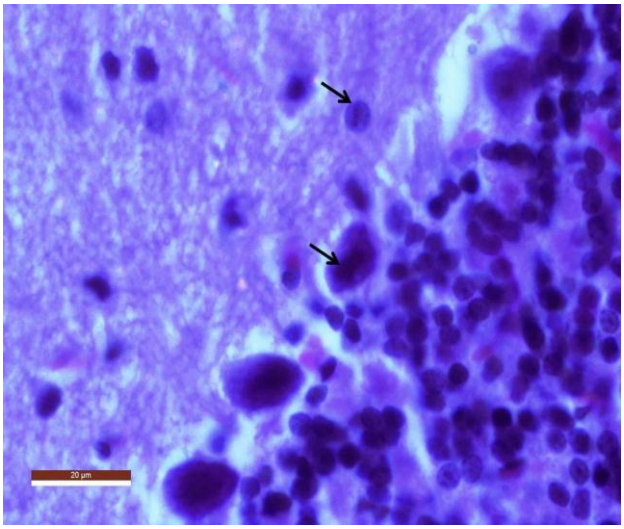

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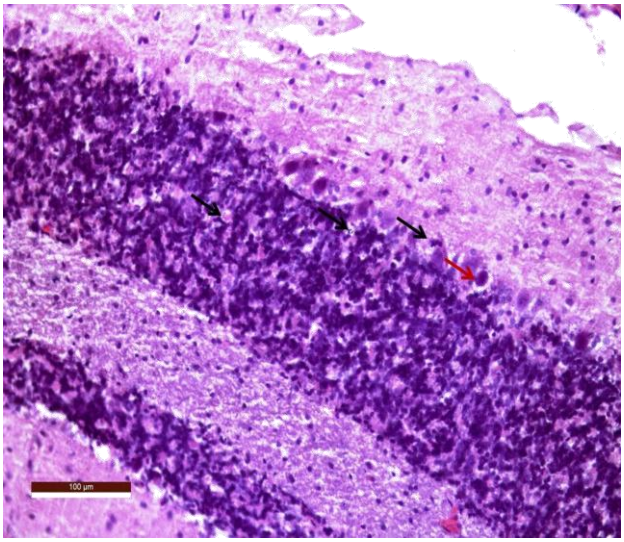

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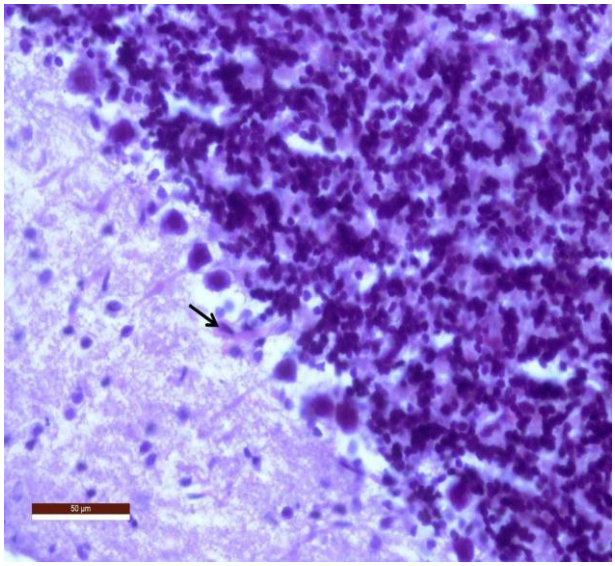

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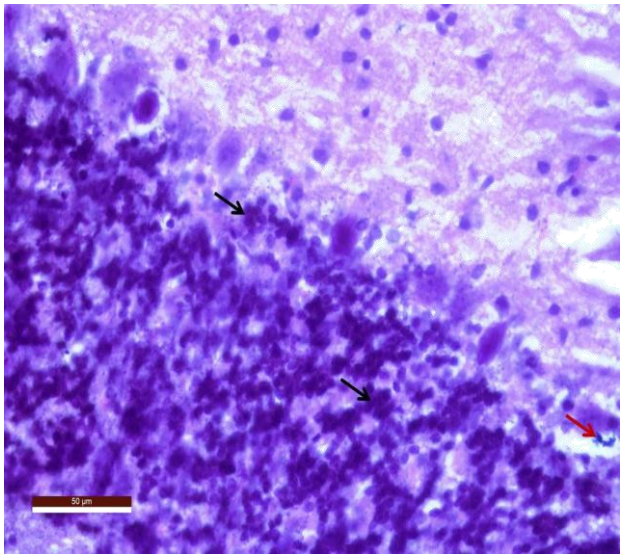

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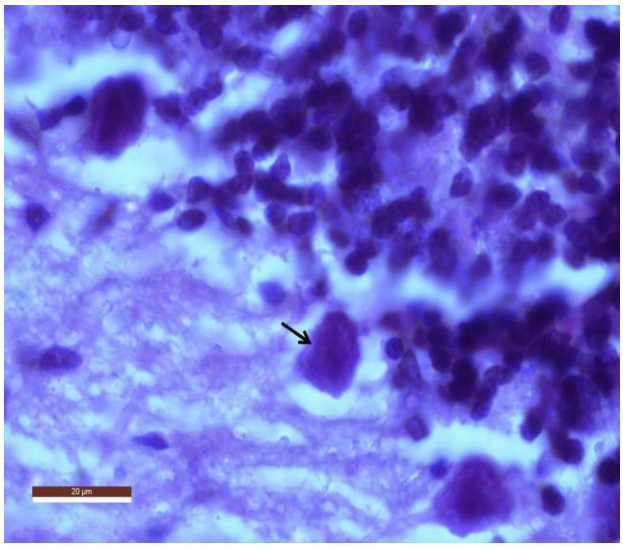

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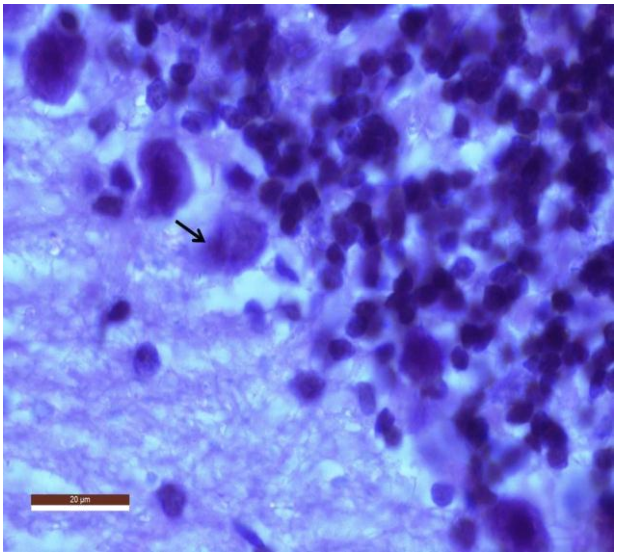

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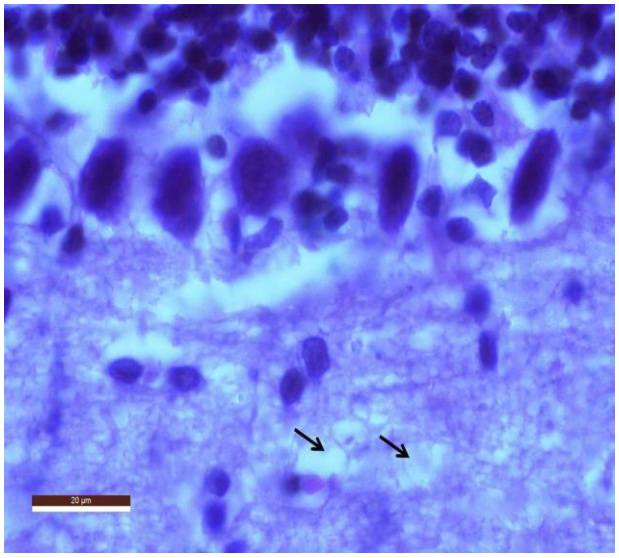

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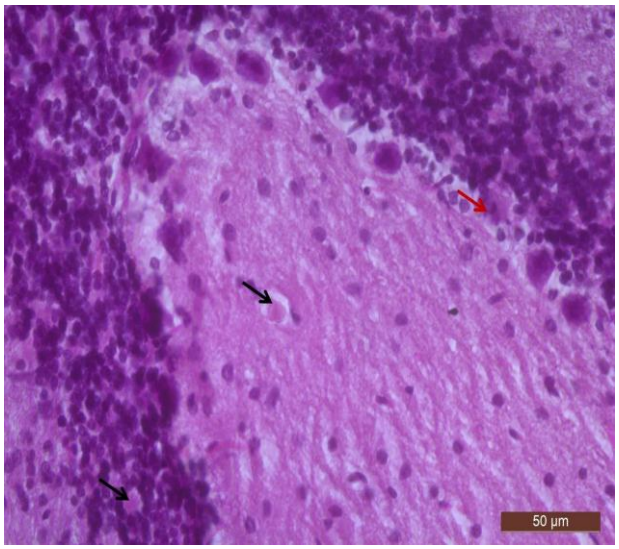

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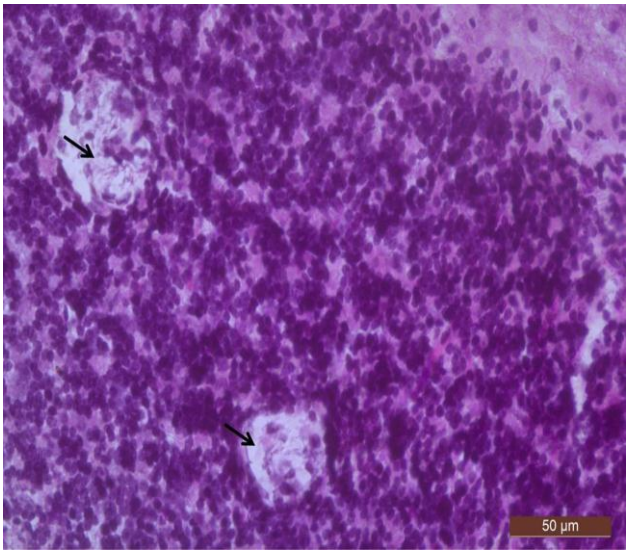

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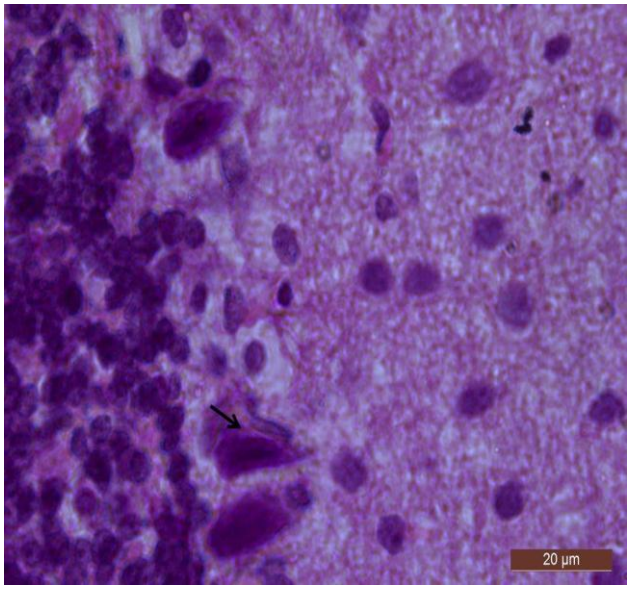

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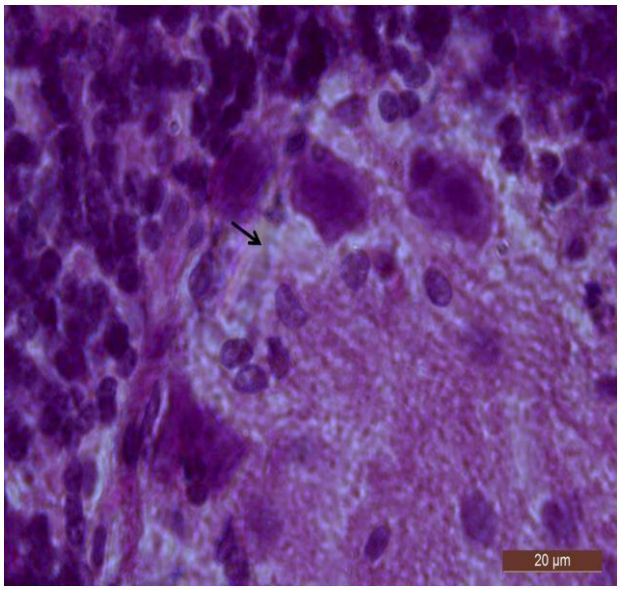

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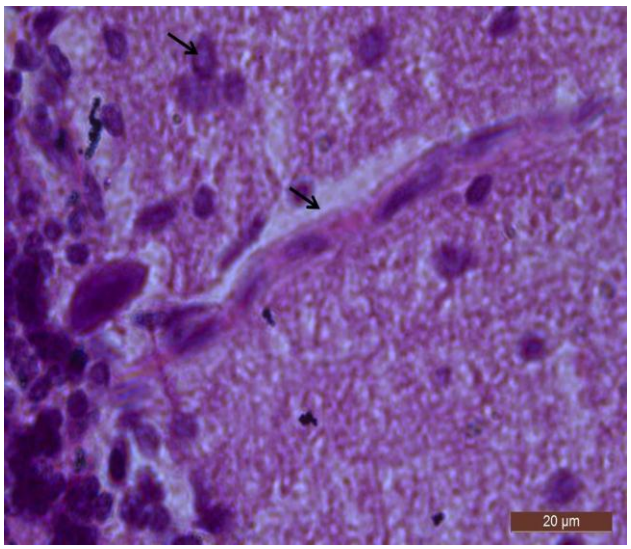

Figure 18.

DISCUSSION

In the present study, cerebellum of rat treated with different concentrations of fluoride, exhibited neuropathological alterations in the form of pyknosis, accumulation of granular cells, degeneration and cellular chromatolysis in the granule cells. These results are in agreement with the findings of earlier reports of Basha *et al*. (2011) who noted presence of eosinophilic Purkinje cells, degenerating neurons, decreased granular cells, gap/space between Purkinje neurons and granular cells, and vacuolations in cerebellum. Akinrinade *et al*. (2013) reported pyknotic nuclei, appearance of vacuolar spaces as an indication of cellular degeneration and ghost like appearance of cells in the cerebellum of fluoride treated rat.

The findings of present study are comparable with the study of Liu (1989) who observed variation in neuronal density and mild degeneration of organelles in nerve cells in the 60 ppm fluoride treated rat. Mullenix et al. (1995) found that fluoride level was higher in cerebellum, hippocampus and basal ganglion of rat exposed to 100 ppm fluoride in drinking water for 6 weeks compared to control. Isaacson *et al*. (1997) observed neuronal abnormalities in the sodium fluoride treated animals especially in deeper cell layer that include distortion of cells, cell losses in particular brain regions, and vascular inclusions. Trabelsi *et al*. (2001) in their study noted increase in cell death, a decrease in the number of granular cells and poor differentiation of Purkinje cell layer in 0.5 g/L fluoride treated rats. They postulated that the destruction of granular layer could be a consequence of fluoride interfering in the mitosis of these cells, and the loss of granular cells in these animals might have resulted due to lack of proper contact between granular cells and Purkinje cells.

Fluoride accumulation has been observed in the brain of experimental animals exposed to chronic high fluoride intake, and this accumulation increased as the drinking water fluoride increases. The neurotoxic changes in the present study are in accord with our earlier study, (Shashi, 2003) in which neurodegenerative changes in Purkinje neurons of brain of rabbits, treated with 5 to 50 mg sodium fluoride/kg body weight/day for 16 weeks, occurred in the form of chromatolysis and disintegration of nuclei, and in some cells the nucleus was displaced to the periphery or at the base of axon. It was shrunken, pyknotic and hyperchromatic. The neuronal loss was accompanied by increased numbers of glial cells. Among the remaining neurons, pear shaped or ballooned forms were frequently prominent. The Nissl substance underwent various degree of change. Shashi and Sharma (2008) reported adenocarcinomatous islands neurons with globose type tangle inside perikaryon in cerebellar cortex. Hypertrophy of neurons in granular layer and atrophy in neurons in molecular layer was observed. They reported disorganization of neuroglial cells with disintegrated and dot like nuclei in brain of rat. Purkinje

cells appeared shrunken and deeply stained with multilayer disposition (El-Dien *et al*., 2010).

The present study demonstrated appearance of spindle and crescent shaped distorted Purkinje neurons, atrophied and hyperatrophied dot like nucleus, neurofibrillary tangles in the cerebellar cortex. Presence of neuronophagia, loss of Nissl substance, and perivascular edema around constricted blood capillaries were observed and are in agreement with earlier findings of Ge *et al.* (2005) and El-Sherif and El-Kholy (2015) which reported absence of nuclei in many nerve cells, various degree of decrease in the Nissl substance, and elongated dendrites along with congested dilated blood vessel surrounded by a wide perivascular space.

The decline in the number of Purkinje neurons seen in present investigation is in harmonious with the findings of Hamid *et al.* (2013), who observed neurodegenerative changes in the form of decrease in size and number of neurons in all the regions of cerebellar cortex, decrease in number of the cells of molecular layer and Purkinje cell layer, and increase in the density of granular cell layer with dense staining.

The neuropathological abnormalities in cerebellar cortex of this study clearly reveal that the fluoride may be playing important role as causative agent for the neuronal cell damage and disruption of cerebellum structural integrity.

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