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Neurotoxicity induced by Fluoride in Rat Cerebral Cortex

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A B S T R A C T

Fluorosis, as a consequence of exposure to high amount of fluoride is a serious public health problem in many parts of world. Fluoride can cause severe damage to the dental and skeletal systems, and is known to be an endogenous neurotoxin. In the present study, Wister albino rats of weighing100-200 gwere given 100, 200 and 300 ppm of sodium fluoride per kg body weight per day for forty conjunctive days while control were given 1 ml double distilled deionized water per kg body weight per day for same duration. The rats were sacrificed under ether anesthesia after fluoride treatment and cerebrum was carefully removed and fixed in Bouin's fluid for neuropathological examination. Neuropathological examinations of cerebrum in fluoride treated rat revealed that some pyramidal neurons showed chromatolysis and were shrunken with vacuolation around them. The nuclei of many pyramidal neurons were eccentric in neuroplasm, irregular and spindle shaped, others were exhibiting necrosis and some presented dark constricted dot like structure. In few pyramidal neurons, the nuclei appeared as eosinophilic or red. Some pyramidal neurons in certain region showed hyperchromatic and hypertrophic nucleus that was filling entire neuroplasm. The granule cells were aggregated in the form of clumps, and neuropil exhibited heterogeneous acidophilic masses containing fragments of nuclei and clear halos. Many granule cells were swollen in shape and size while some appeared darkly stained with dot like shrunken nuclei and empty spaces around them. In cerebral cortex of fluoride treated rat, the blood capillaries were congested with narrow lumen and perivascular empty spaces.

Introduction

Fluoride is probably the first inorganic ion which drew attention of the scientific world for its toxic effects and now the fluoride toxicity through drinking water is well-recognized as a global problem. If fluoride is consumed in high quantities, it can cause severe damage to most tissues including primarily the dental and skeletal systems. In the last several decades, effects of fluoride on the brain and its activity have become the subject of considerable interest in the field of fluoride toxicity. Fluoride crosses the blood brain barrier causes neuro-degeneration (Lubkowska *et al*., 2004). Li *et al*., (1995) observed adverse neurological effects in the brain of humans with exposure to fluoride. In experimental studies, fluoride accumulation was observed in the brain of

animals exposed to chronic fluoride intake and this accumulation increased as drinking water fluoride content increased (Mullenix *et al*., 2005). It was reported that long termintake of high level of fluoride in human caused neurological complications such as paralysis of limbs, vertigo, spasticity in extremities and impaired mental acuity (Lu *et al*., 2000).The cerebral cortex comprises most of the motor areas and important vital centers. It is considered to be responsible for programming of complex motor activities. Hence, the current study was aimed to find out the neuropathological effects of different concentrations of sodium fluoride in cerebrum of albino rats.

Material and Methods

Experimental animals

Young and healthy Wistar albino rats weighing 100-200 g were housed in polypropylene cages with stainless steel grill tops and bedded with paddy husk. They were kept under standard laboratory conditions maintained at 25±2^{o} C and 12 hours light and dark cycle and were fed on standard pellet diet obtained from Hindustan Lever Limited, Mumbai, India. Water was given *ad libitum*. The animals were acclimatized to the laboratory conditions for two week prior to the experimentation. The experimental protocol was approved by Institutional Animal Ethics Committee, Punjabi University, Patiala (Approval no.107/99/CPCSEA-2012-10).

Experimental design

Rats were weighed and randomly divided in four groups with six rats per group. The administration of sodium fluoride lasted for forty consecutive days which was done via oral gavage. Group I was given 1 ml double distilled water/kg body weight/day and was kept as control group, while remaining groups II, III and IV were treated with 100, 200 and 300 ppm sodium fluoride per kg body weight per day respectively.

Neuropathological study

The rats were fasted overnight and sacrificed under ether anesthesia after 40 days fluoride treatment. The cerebrum was carefully removed and fixed in Bouin's fluid for neuropathological examination. The fixed tissue were 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 then 7 µm thin serial sections were cut with rotary microtome and stained with hematoxylin and eosin (Drury and Willington, 1967) and examined under microscope (Leica DM 2000). The photomicrographs of cerebrum tissue section were clicked with camera (Leica DFC 450 C) fitted on microscope system.

Results and Discussion

In control rat, the cerebral cortex contained pyramidal neuron of normal shape and size The nucleus was large and intensely stained. The granule cells were scattered in the neuropil. The fusiform cells were oriented at right angle to the cerebral cortex and axons were arising from both side of the cell body and passing superficially (Fig. 1).

In cerebral cortex of the rats, treated with 100 ppm of sodium fluoride, there was disorganization of granular cell layer. The granule cells were accumulated in the form of clumps. Some pyramidal neurons showed chromatolysis (Fig. 2) and were shrunken with vacuolation around them (Fig. 4). The nuclei of some pyramidal neurons were eccentric in neuroplasm and others were

showing necrosis. Some granule cells were swollen in shape and size (Fig. 5). At some location, neuropil exhibited heterogeneous acidophilic masses containing fragments of nuclei and clear halos (Fig. 3).

In rats treated with 200 ppm of sodium fluoride, the blood capillaries were constricted with narrow lumen and perivascular empty spaces were observed in the cerebral cortex. The deeply stained atrophied nuclei in some glial cells were visible (Fig. 6). At some locations in the neuropil, granule cells showed irregular arrangement with deeply stained, hypertrophied hyperchromatic nuclei with empty spaces around granule cells (Fig. 7). Some glial cells appeared as darkly stained with dot like shrunken nuclei and empty spaces around them (Fig. 8). Some pyramidal neurons showed necrosis and nuclei were in the form of dark constricted dot like structures (Fig.9). There were some pyramidal neurons which were irregular in shape and size with darkly stained spindle shaped nuclei and some nuclei were eccentric inside the neuroplasm (Fig. 10). In some neurons, the nuclei appeared as eosinophilic or red. The neuronal degeneration and hypertrophy with perineuronal empty spaces were visible in the pyramidal neuron (Fig. 11). In cerebral cortex, some area showed necrosis with complete disintegration of neuropil accompanied by loss of glial cells (Fig.12).

The pyramidal neurons in some area showed hyperchromatic and hypertrophic nucleus that was filling entire neuroplasm (Fig. 13). There were visible rounded fluid filled areas showing gliosis having debris of glial cells. The pyramidal neurons were distorted with spindle shape structure having elongated nucleus (Fig. 14).

In rats treated with 300 ppm of sodium fluoride, cerebral cortex contained the glial cells that were small, deeply stained and dot like structure with vacuolated areas around them (Fig. 15). The neuropil showed degeneration in the form of spiral structure surrounded by microglial cells (Fig. 16). At some locations eosinophilic neurons were visible in neuropil. Area of neuroplasm of some neuron was covered by darkly stained swollen large sized nucleus. Clear halos were visible around some neurons (Fig. 17). A few neurons exhibited degeneration at some locations in neuropil (Fig. 18). In some pyramidal neurons distorted nuclei were shifted to periphery. Chromatolysis visible in some granule cells (Fig. 19).

Extensive epidemiological and experimental studies have established that the biological responses of animals to fluoride are related to dosage and other factors that influence the animal's physiological and anatomical responses. Evidence that fluoride crossed the blood brain barrier (Lubkowska *et al*., 2004) raised the possibility that fluoride could affect the structure and function of the central or peripheral nervous system.The histology of cerebral hemisphere was altered by sodium fluoride (Shah and Chinoy, 2004).The observations of present work revealed marked alternations in the neuropathology of the cerebral cortex of fluoride treated rats.

Many granule cells were swollen in shape and size while some appeared darkly stained with dot like shrunken nuclei and empty spaces around them. Some pyramidal neurons showed chromatolysis (Trivedi *et al*., 2012), and were shrunken with vacuolation around them. These results are in accord with the results of Ge *et al*.(2005), Fattah *et al*., (2010), Hamid *et al.,* (2012) and Shashi and Sharma (2015), who documented distinct morphological alterations in the brain including effects on neurons of the cerebrum.

Fig.1 T. S of cerebral cortex of control rat showing normal shape and size of pyramidal neuron with dendrites, and granules cells. Fusiform cell oriented at right angle to the cerebral cortex, axons arise from the side of the cell body and pass superficially. H&E, X1000.

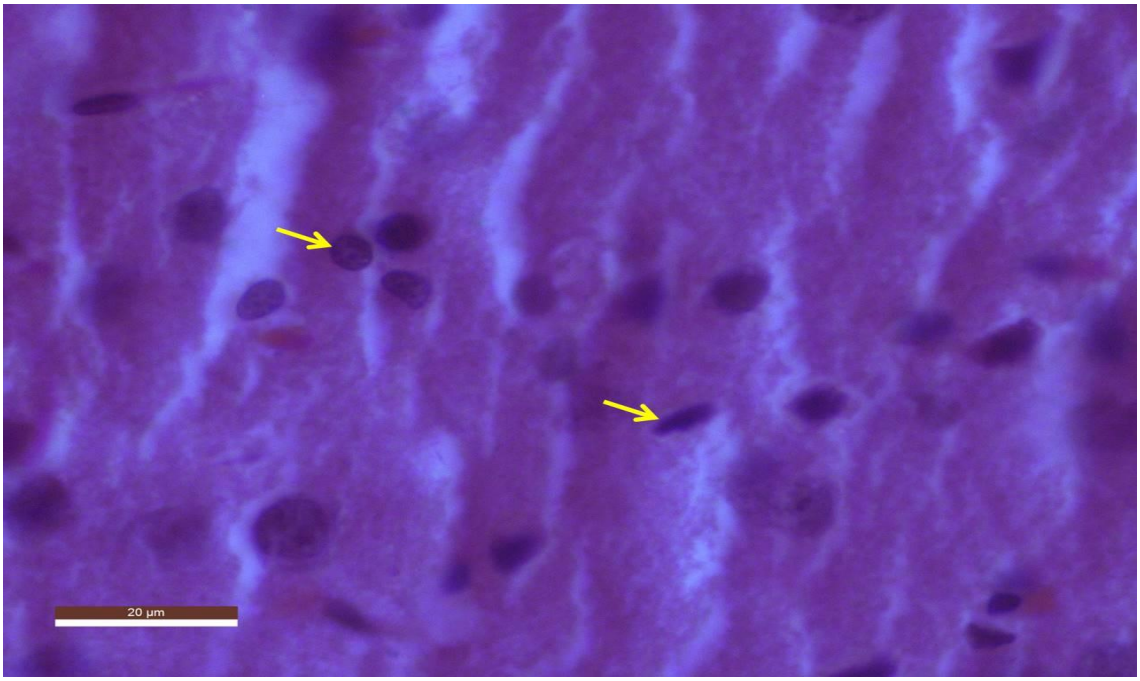


Fig.2 T. S of cerebral cortex of rat treated with 100 ppm sodium fluoride showing accumulation of granular cells in the form of clump granule cells (black arrow) and chromatolysis of pyramidal neurones (yellow arrow). H&E X400.

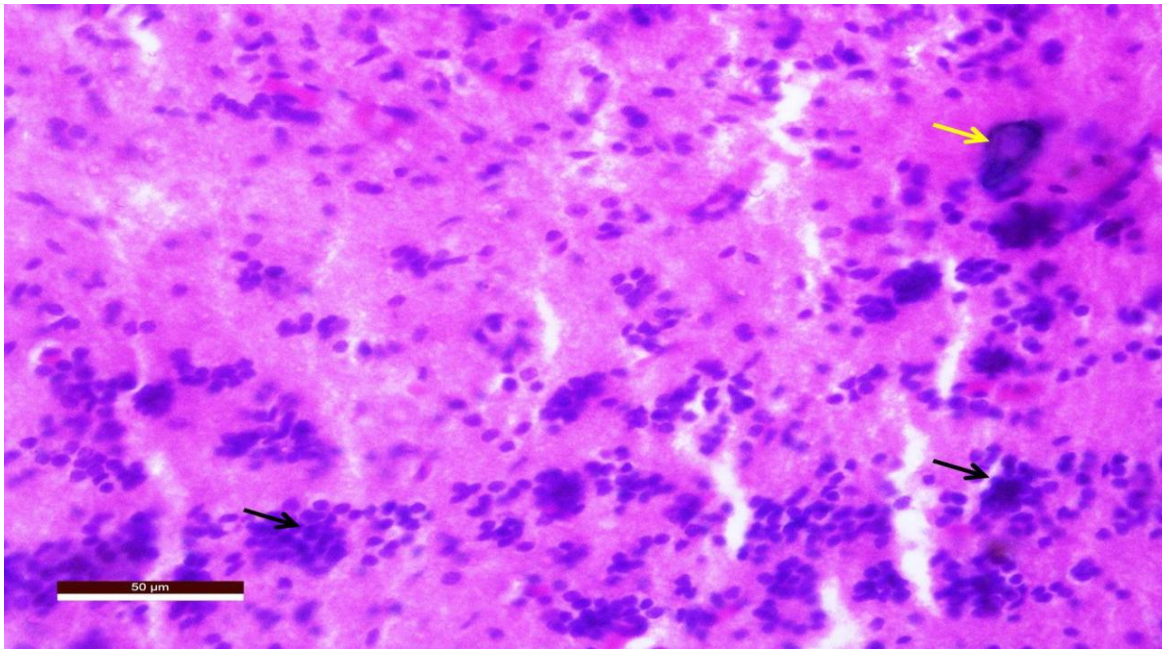


Fig.3 T. S of cerebral cortex of rat treated with 100 ppm sodium fluoride showing vacuolation around shrunken pyramidal neurons. H&E, X1000.

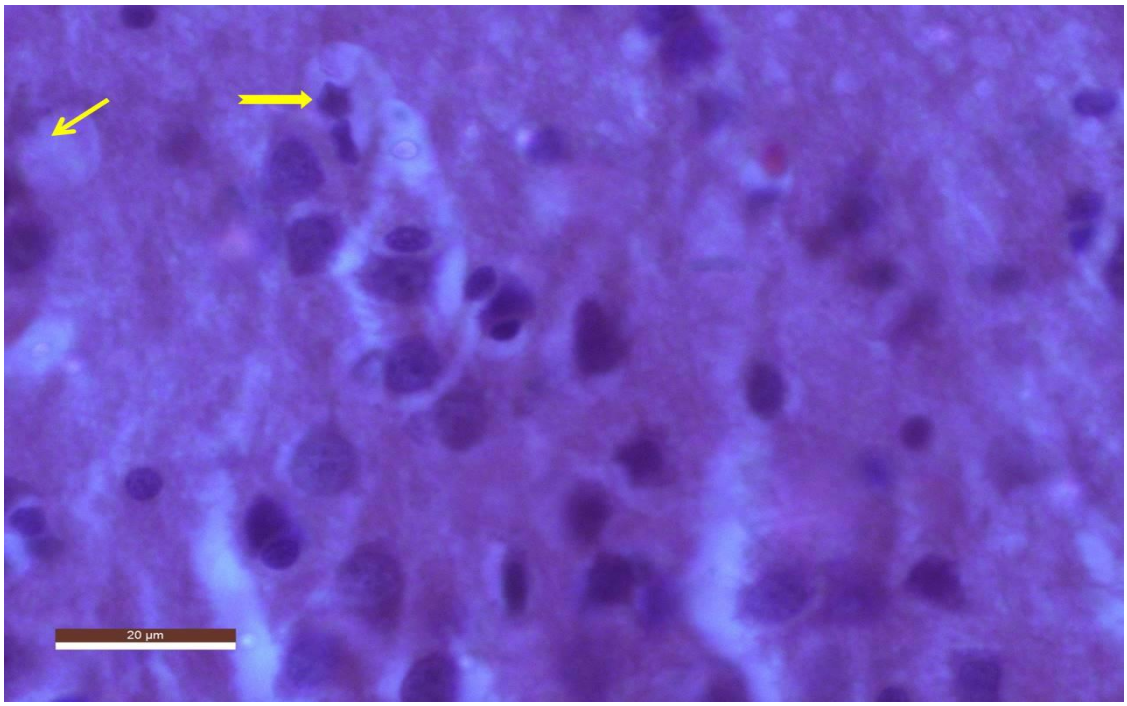


Fig.4 T. S of cerebral cortex of rat treated with 100 ppm sodium fluoride showing swollen granule cells. H&E, X1000.

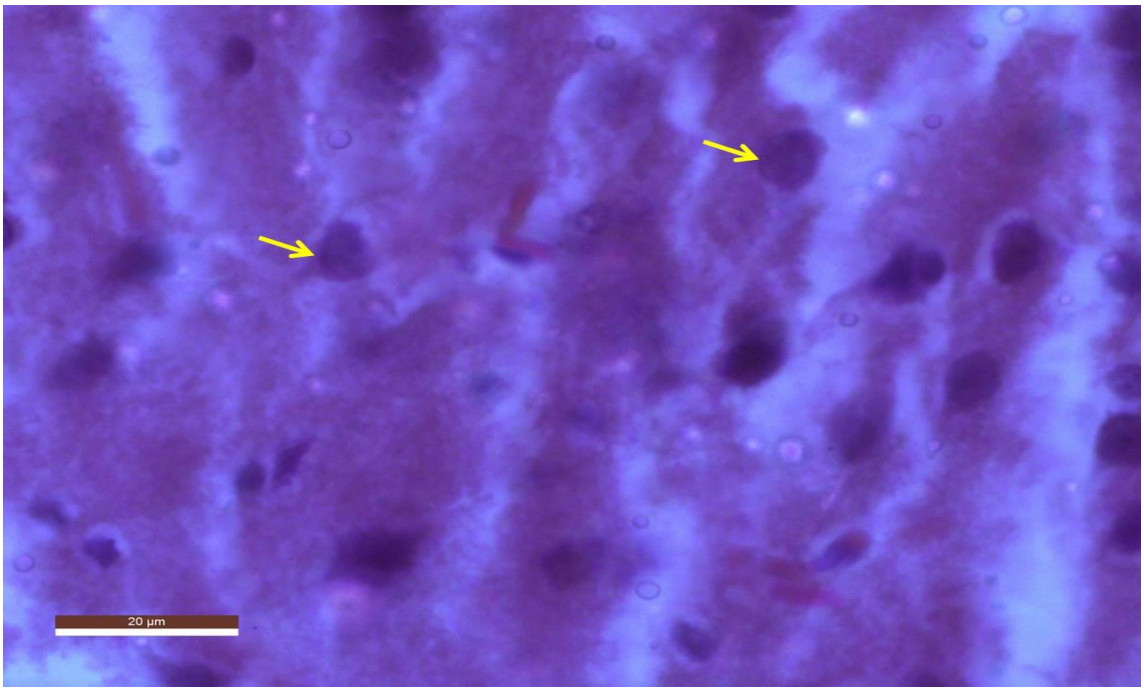


Fig.5 T. S of cerebral cortex of rat treated with 100 ppm sodium fluoride showing spongiosis of neurons and heterogeneous acidophilic masses containing fragmented nuclei and clear halos. H&E, X400.

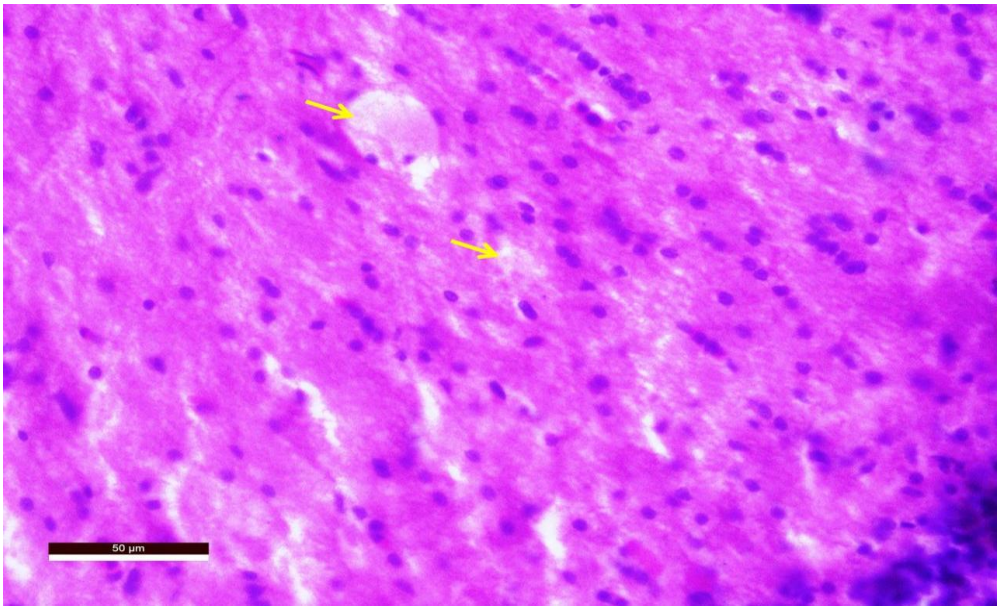


Fig.6 T. S of cerebral cortex of rat treated with 200 ppm sodium fluoride showing constricted blood capillaries with perivascular empty spaces (arrow with basal notched head). Some neurons were deeply stained, hypo atrophied nucleus surrounded by clear halos (arrow with triangular head), and some pyramidal neurons were hypertrophied as compared to control (arrow with notched tail). H&E, X400

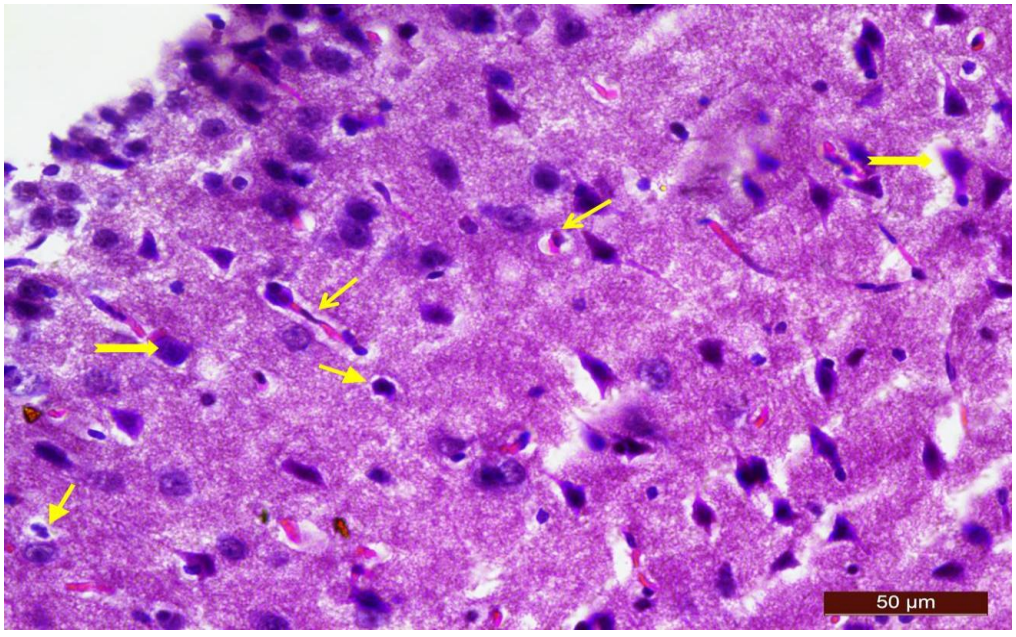


Fig.7 T. S of cerebral cortex of group III rat treated with 200 ppm sodium fluoride showing irregular arranged granule cells with deeply stained large in size, having hyperchromatic nucleus with pericellular halos. H&E, X400.

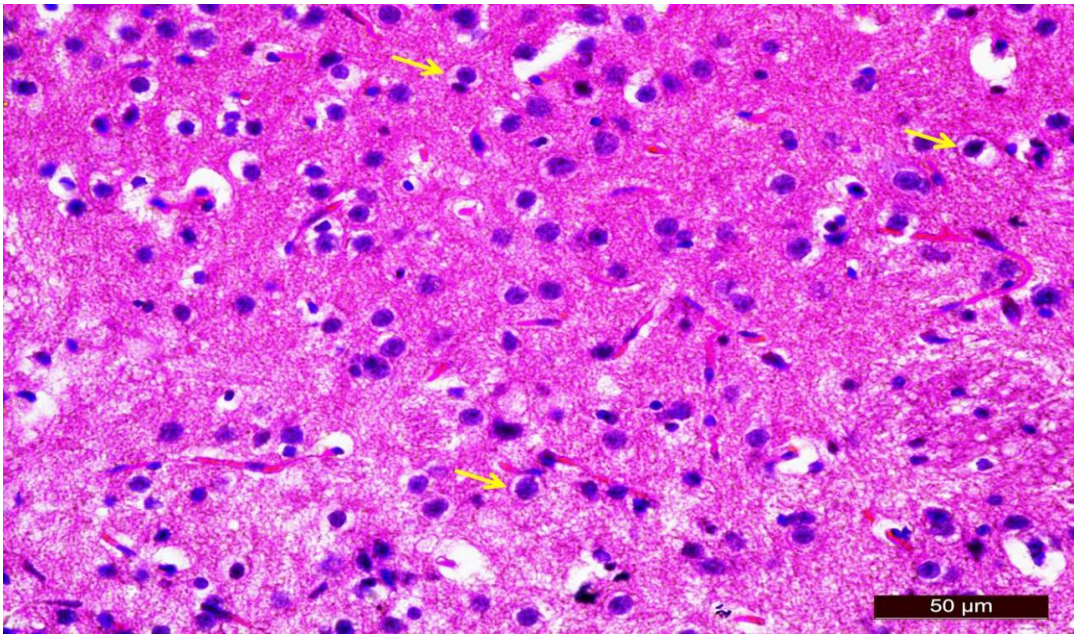


Fig.8 T. S of cerebral cortex of rat treated with 200 ppm sodium fluoride showing glial cells with shrunken, darkly stained and dot like appearance of nucleus surrounded by empty spaces. H&E, X400

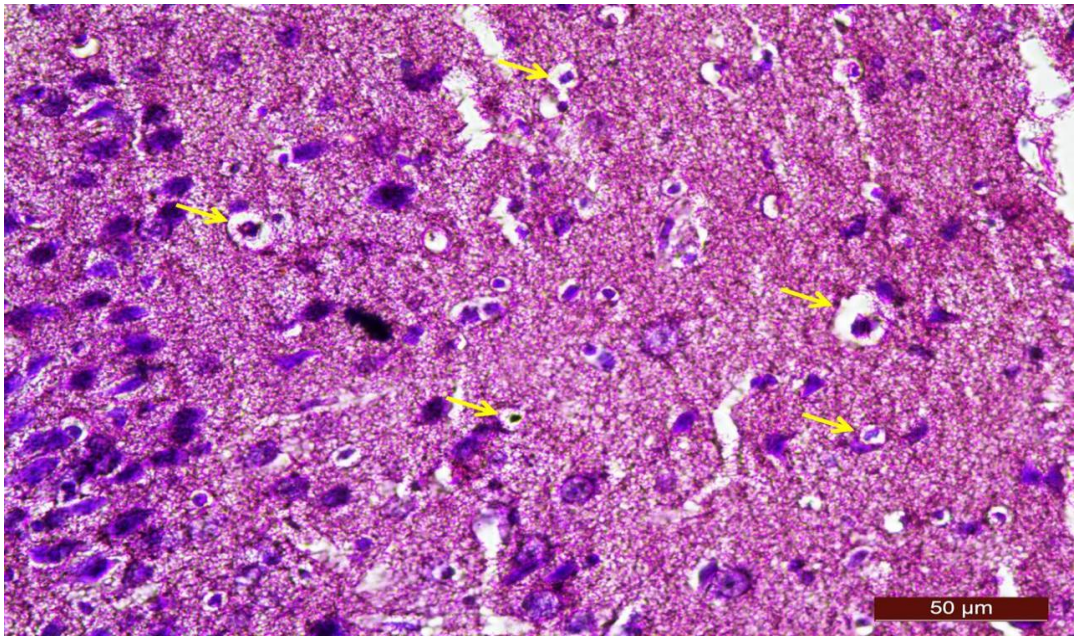


Fig.9 T. S of cerebral cortex of rat treated with 200 ppm sodium fluoride showing pyknosis in granule cells (arrow with notched tail) and some neurons were seen with constricted, dot like appearance and deeply stained nucleus (arrow). H&E, X400.

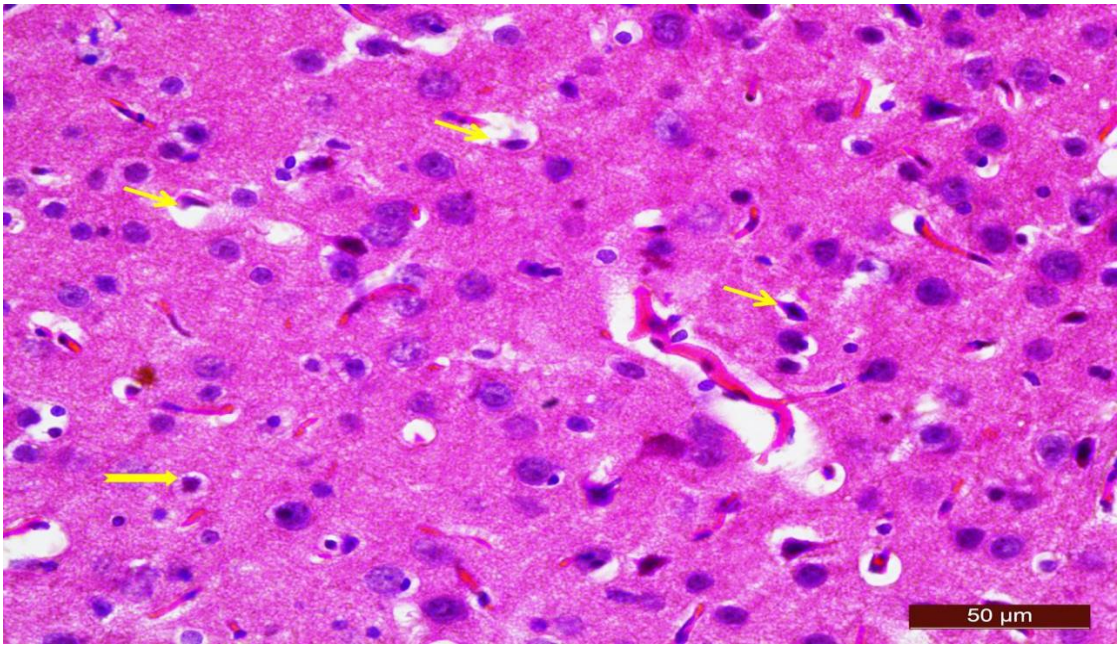


Fig.10 T. S of cerebral cortex of rat treated with 200 ppm sodium fluoride showing irregular shape and size of pyramidal neurons with darkly stained, spindle shaped, eccentric nucleus. H&E,X400.

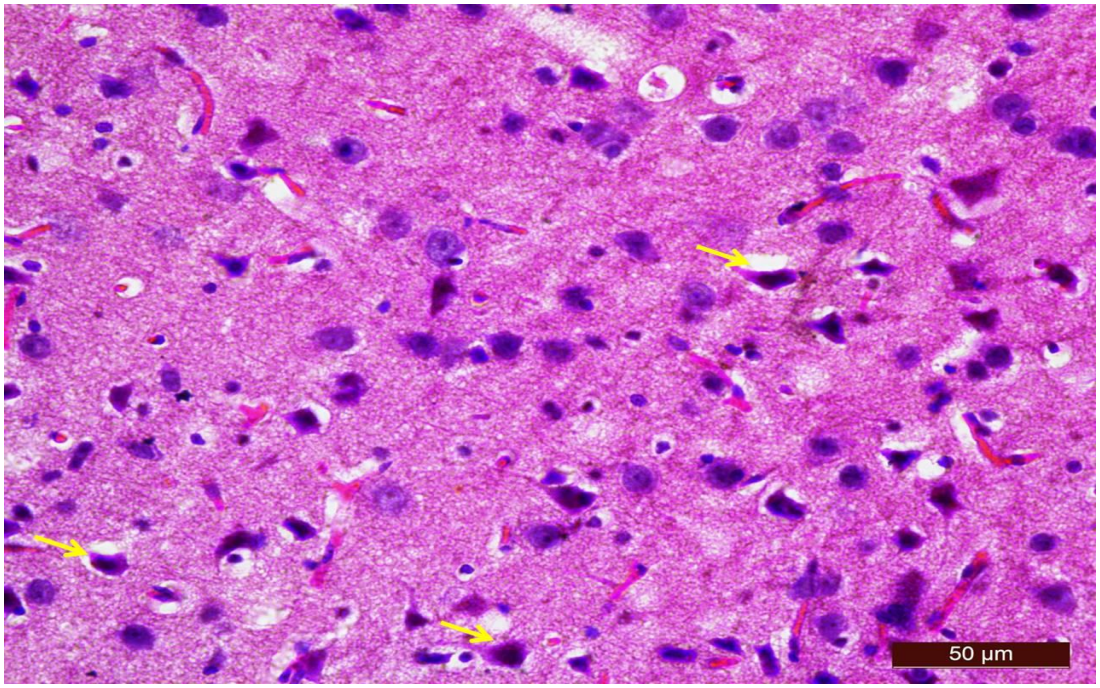


Fig.11 T. S of cerebral cortex of rat treated with 200 ppm sodium fluoride showing eosinophilic neurons (black notched arrow), hypertrophied pyramidal neurons (yellow arrow) and degenerating neurons with perineuronal spaces (black arrow). H&E, X1000.

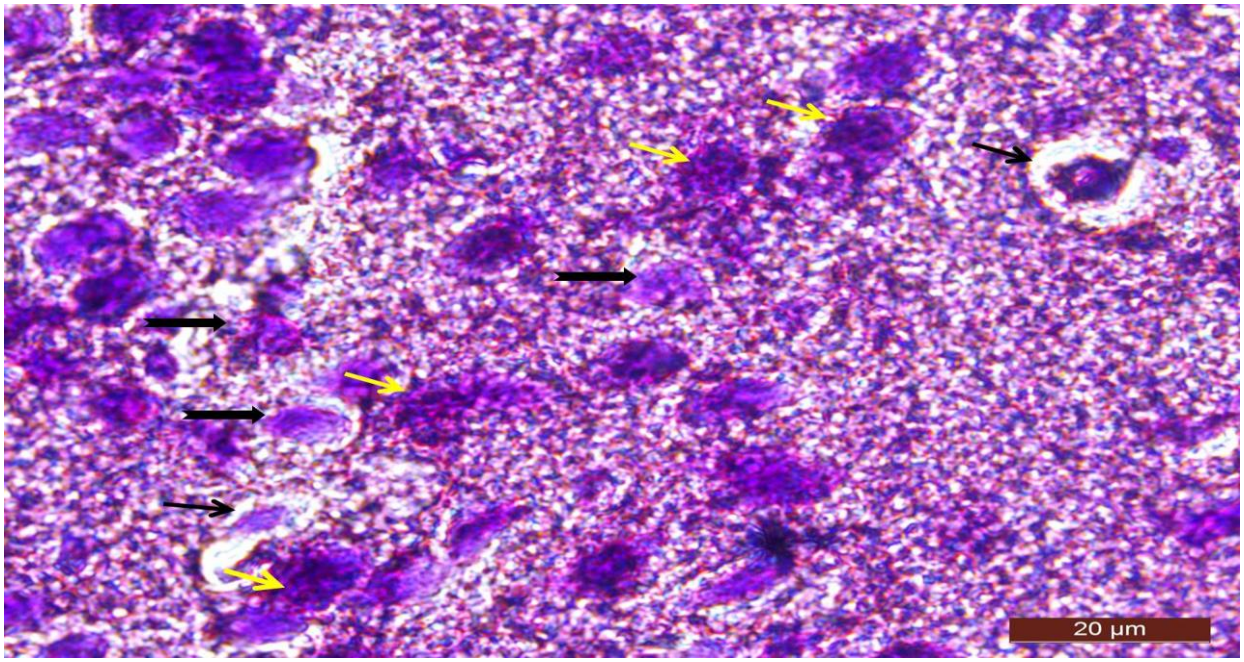


Fig.12 T. S of cerebral cortex of rat treated with 200 ppm sodium fluoride showing highlnecrotic areas with complete disintegration of cerebral components accompanied by loss of glial cells. H&E, X1000

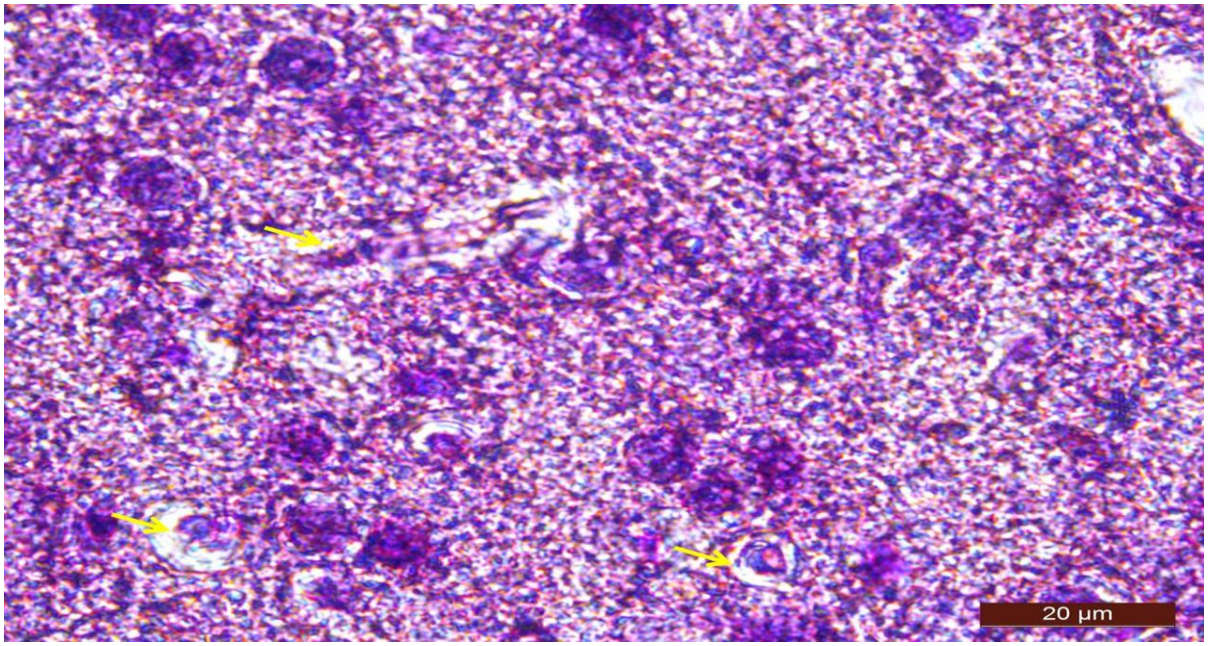


Fig.13 T. S of cerebral cortex of rat treated with 200 ppm sodium fluoride showing hyperchromatic pyramidal neuron with hypertrophied nucleus that filled in the neuroplasm, and distorted shape with the empty spaces both side of the neuron. H&E, X1000

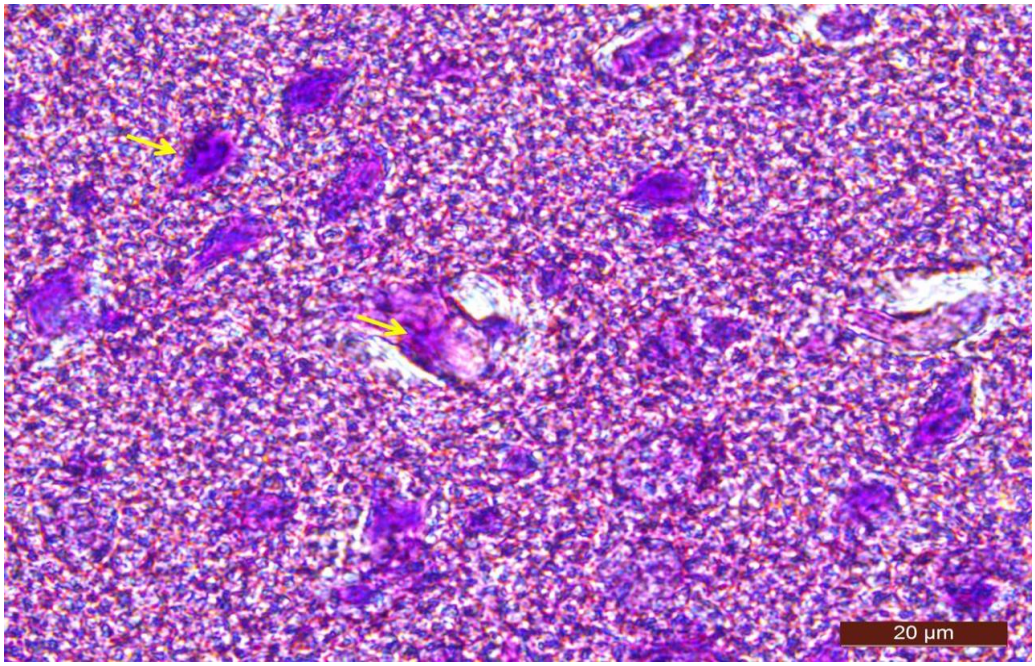


Fig.14 T. S of cerebral cortex of rat treated with 200 ppm sodium fluoride showing heterochromatic, rounded, fluid filled areas having debris of glial cells. Spindle shaped neurons with elongated nucleus was present. H&E, X1000.

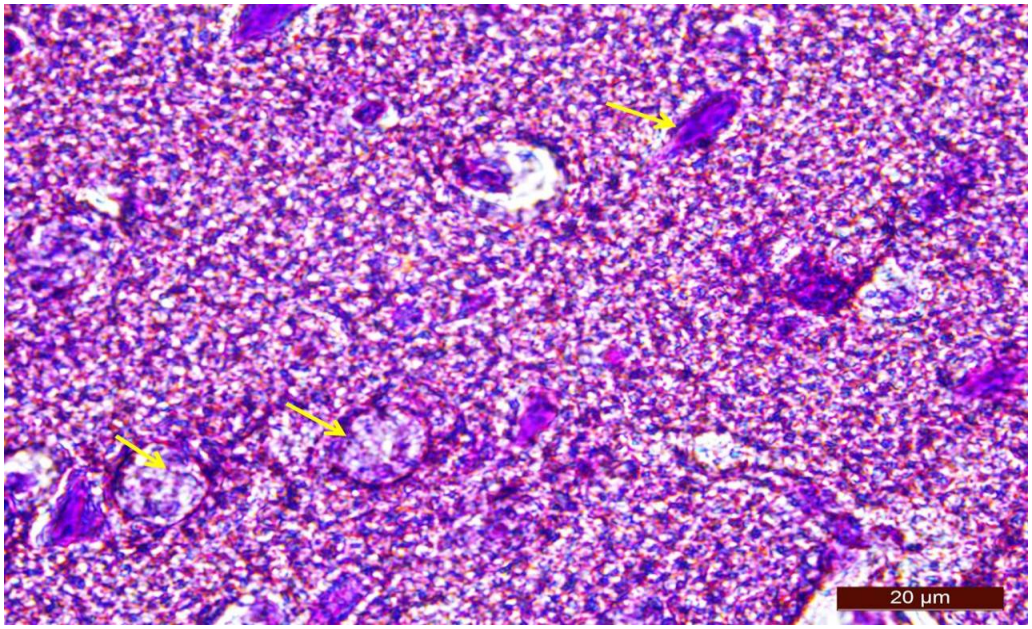


Fig.15 T. S of cerebral cortex of rat treated with 300 ppm sodium fluoride showing small, deeply stained dot like nucleus in the glial cells and vacuolated area around glial cells. H&E, X200

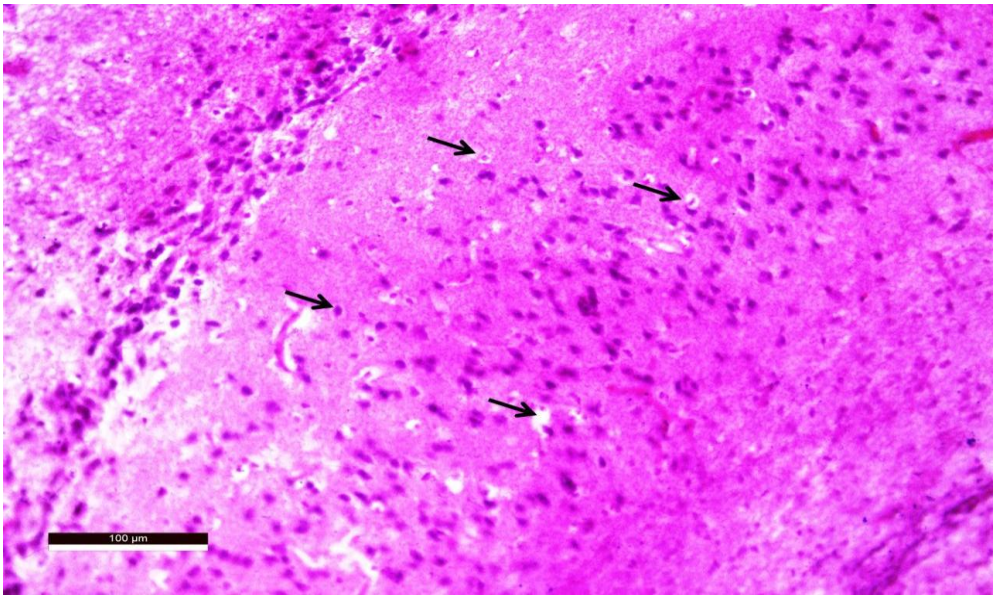


Fig.16 T. S of cerebral cortex of rat treated with 300 ppm sodium fluoride showing degeneration of neuropil in spiral form, surrounded by microglial cells, and some glial cells appeared dark stained in the neuropil. H&E, X400

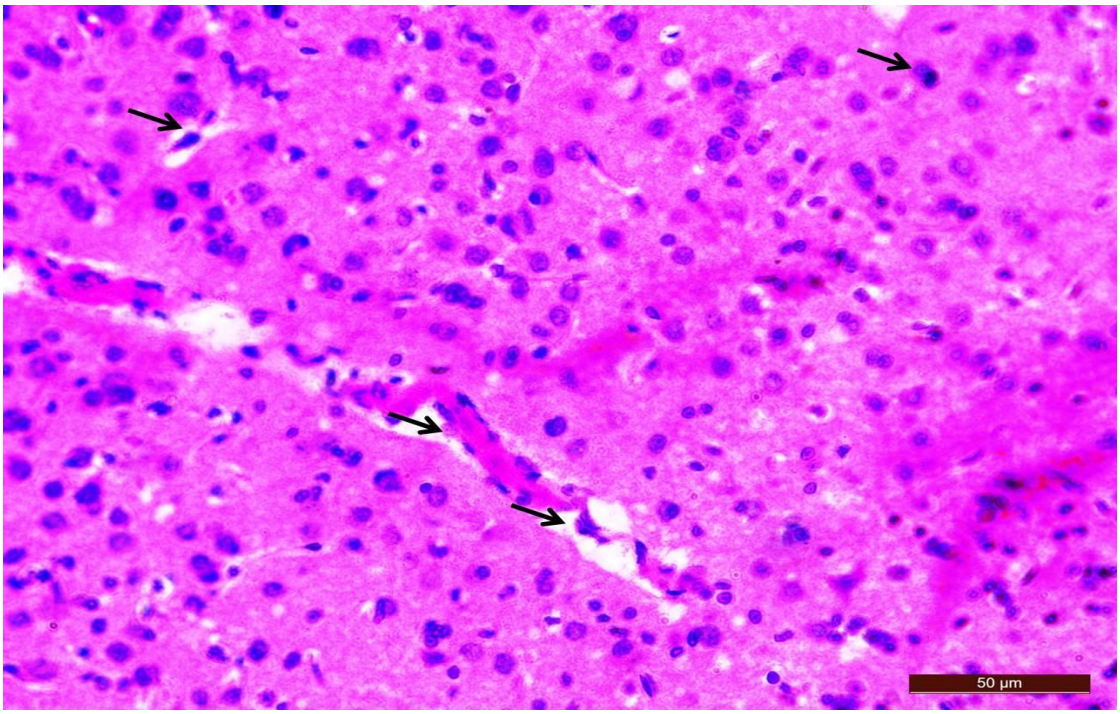


Fig.17 T. S of cerebral cortex of rat treated with 300 ppm sodium fluoride showing distorted, eosinophilic pyramidal neurons with halos and some neurons with dark staining, hypertrophied nucleus occupying complete area of the neuron. H&E, X1000

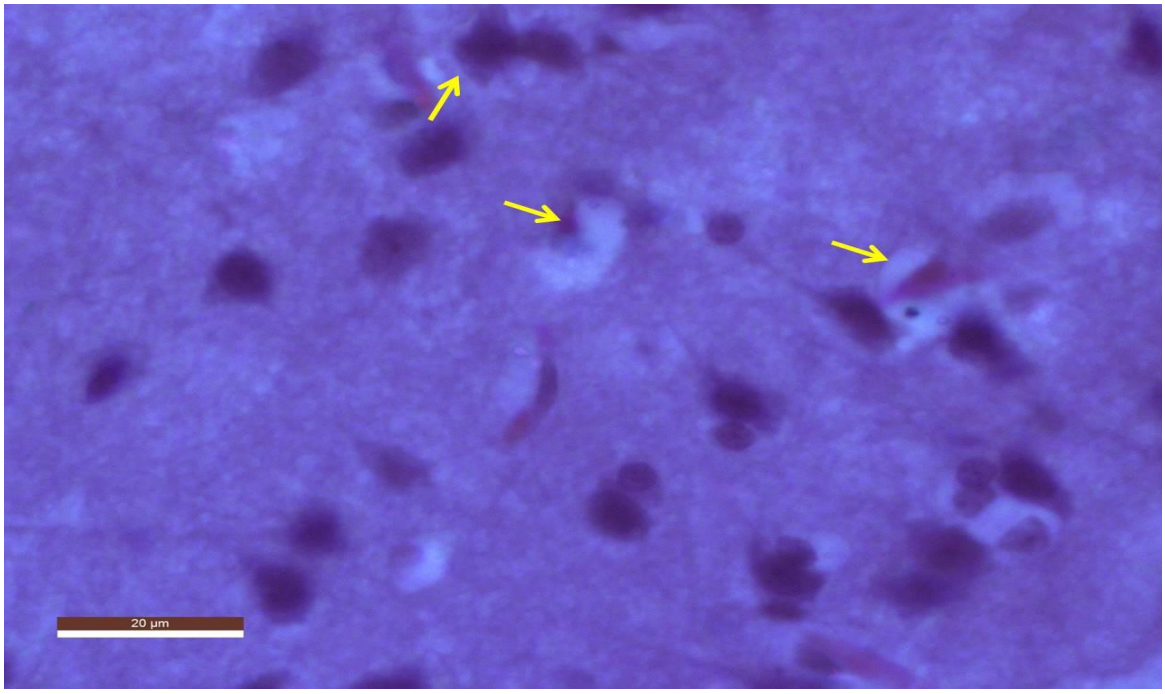


Fig.18 T. S of cerebral cortex of rat treated with 300 ppm sodium fluoride showing degeneration of neurons, H&E, X1000

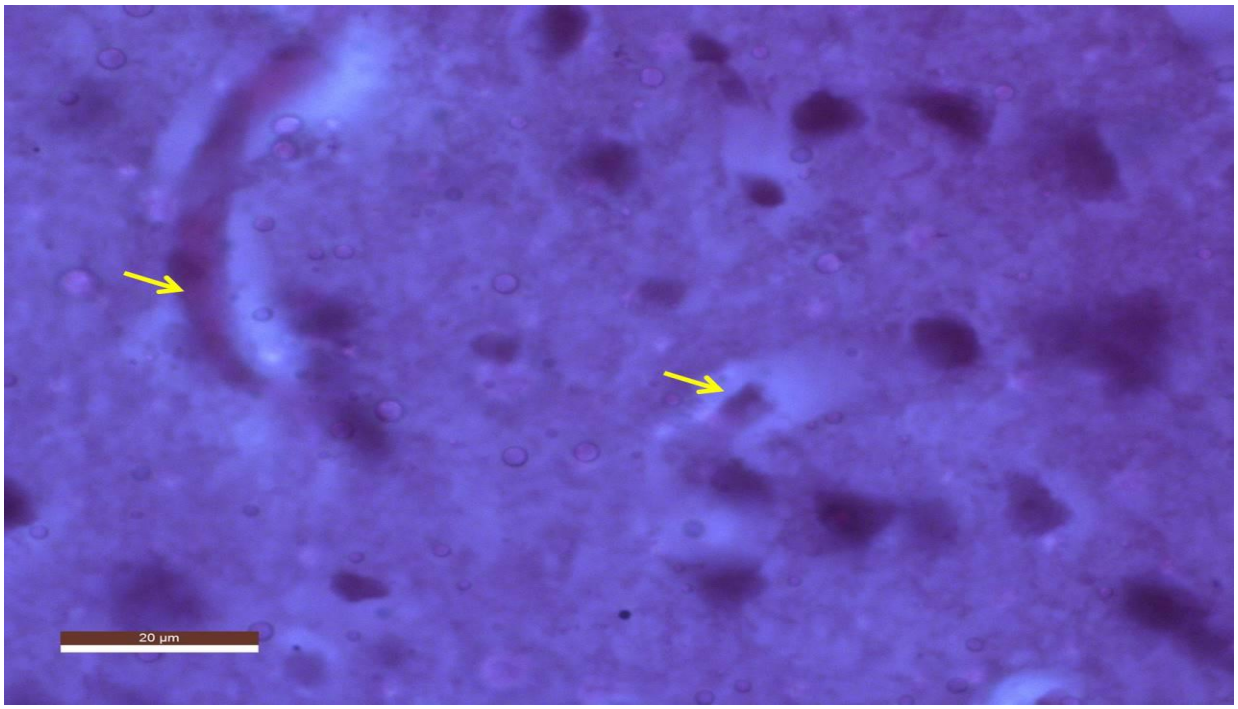
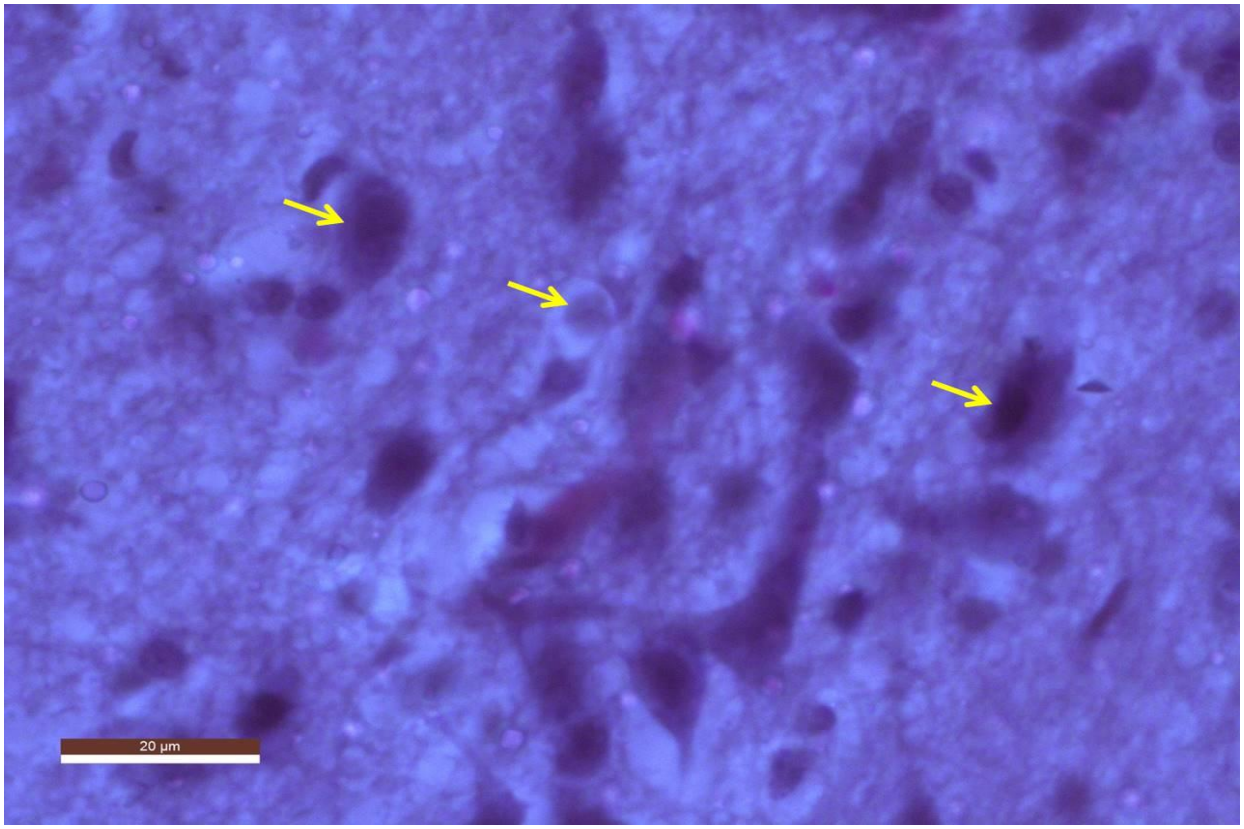


Fig.19 T. S of cerebral cortex of rat treated with 300 ppm sodium fluoride showing chromatolysis of granule cells and displacement of nucleus to the periphery of the distorted pyramidal neurons, H&E, X1000



In other study on amygdala, motor cortex, cerebellum, hippocampus (Shivarajashankara *et al*., 2002), and the cerebrovascular integrity after chronic administrations of sodium fluoride or aluminum fluoride (Varner *et al*., 1998) comparable results was obtained.

Rehman and Nasir (2014) reported a decrease in neuronal density and mitotic figures with pyknosis and vacuolations throughout the cerebrum of rat treated with sodium fluoride which confirms the findings of this study. Presence of eosinophilic pyramidalneurons, degenerating neurons, necrosis, chromatolysis and decreased granular cells in discrete cerebral regions of the fluoride treated groups indicate that there is a tendency for neurone apoptosis in chronic fluorosis in rats, is most evident with changes in neuropathology.

Acknowledgement

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