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Research Article

NEUROPATHOLOGICAL CHANGES IN HIPPOCAMPUS IN ALBINO RAT IN FLUORIDE TOXICITY

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ABSTRACT
The study was carried out to evaluate the neuropathological effect of sodium fluoride in hippocampus of albino rat. Healthy albino rats of Wistar strain weighing 100-200 g were divided in 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 hippocampus were studied for neuropathological abnormalities. In rats treated with 100 ppm sodium fluoride, the hippocampus showed various degree of disarrangement of pyramidal layer. The pyramidal neurons appeared distorted with elongated rod shaped nucleus. Shrunken and darkly stained nuclei were visible in some neurons as compared to control. In rats treated with 200 ppm sodium fluoride, many neurons displayed irregularities in their structure and distribution. A decrease in the density of pyramidal cells in CA1 region was observed. The neuropil displayed various degree of necrosis, granular cells showed vacuolation and disintegration of neuroplasm. In hippocampus of rats treated with 300 ppm sodium fluoride, neurotoxic changes were very much prominent compared to control. Constricted blood capillaries were visible in the neuropil. Some nerve cells contained pyknosed nuclei. The polymorphic layer depicted degeneration and atrophied nerve cells. At some locations, clear halos were visible in neuropil. Some granule cells and pyramidal neurons exhibited chromatolysis. The findings of present study illustrated that fluoride may worsen the cytoarchitectural arrangement in the hippocampus by altering the structure of pyramidal neurons and neuroglia. The nuclei of pyramidal neurons in hippocampus may be the main organelle that gets affected and starts depicting variation in size and structure in different concentration of sodium fluoride.

Keywords: *Albino Rat, CA1, CA2, CA3, Hippocampus, Pyramidal Neuron, Sodium Fluoride*

INTRODUCTION
High levels of fluoride in drinking water have become a potential health hazard all over the world. Ingestion of fluoride induces adverse effects in teeth and skeletal system as well as the structure and functions of non-skeletal systems such as muscle (Shashi and Rana, 2016), kidney (Karaoz *et al.,* 2004), liver (Manna *et al.,* 2007), myocardium (Basha and Sujitha, 2011), thyroid gland (Shashi and Kumar, 2016a) including brain (Shashi, 2003). Excessive fluoride exposure may result in central nervous system dysfunction (Spittle, 1994). Several neurological symptoms have been observed in fluorosis patients (Basak *et al.,* 2016). Scientific studies have shown that excess fluoride in drinking water damages the nervous system. Chronic fluoride exposure is associated with the eventual rise in incidence of neurodegenerative diseases such as Alzheimer's dementia (Blaylock, 2004). The hippocampus is the key region for learning and memory in the brain and has been postulated to be one of the neurotoxic target sites attacked by fluoride (Bhatnagar *et al.,* 2002).
The hippocampus is a major component of the brains of humans and other mammals. It belongs to the limbic system and plays important roles in the consolidation of information from short-term memory to long-term memory and spatial navigation. Like the cerebral cortex, with which it is closely associated, it is a paired structure, with mirror-image halves in the left and right sides of the brain. In humans and other primates, the hippocampus is located inside the medial temporal lobe beneath the cortical surface. The hippocampus has a shape of a curved tube, which has been analogized variously to a seahorse. The hippocampi are vital regions found in each of the cerebral hemispheres. It consists of two interlocking

laminae of gray matter: the Cornu Ammonis (Ammon's horn) and the dentate gyrus. The Cornu Ammonis (CA) is divided into four regions; CAl, CA2, CA3, and CA4 (Hayman *et al.*, 1998).

Fluoride is known to accumulate in various parts of brain especially in hippocampus (Burgstahler and Colquhoun, 1996). Evidence that fluoride crossed the blood brain barrier raised the possibility that fluoride could affect the structure and function of the central or peripheral nervous system. During the past decade researchers all over the world have felt that there is a need to study the effects of fluorides on brain. Thus, the present study elucidates the effect of different doses of fluoride on the neuropathology of hippocampus in albino rats.

MATERIALS AND METHODS

Experimental Animals: Young and healthy Wister 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 hour 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 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 the remaining group II, III and IV was treated with 100, 200and 300 ppm respectively.

Neuropathological Examinations: The rats were fasted overnight and sacrificed under ether anesthesia after 40 days of fluoride treatment. The hippocampus was carefully removed and fixed in Bouin's fluid, 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 (Drury and Wellington, 1967) and examined under microscope (Leica DM 2000) and subsequently microphotographs were taken with camera (Leica DFC 450 C) fitted on research microscope.

RESULTS AND DISCUSSION

Results

In control rat hippocampus, distinct four regions viz. CA1, CA2, CA3 and CA4 and Dentate gyrus (DG) regions with normal neuronal architecture were observed (Figure 1). CA1 and CA2 zones comprise of small pyramidal neurons. CA3 and CA4 zones of hippocampi comprise large pyramidal neurons. DG region composed of granular cells. There is a narrow hippocampus sulcus. Hippocampus comprises of molecular layer, pyramidal layer, and polymorphic layers (Figure 2). The pyramidal layer is the principal cell layer. The polymorphic layer consisted of neuronal processes (axons and dendrites), blood capillaries, glial cells, and scattered nerve cells. The pyramidal neurons were normal with triangular, pyramid shaped cell body, nodded axon, multiple branched dendrites with spines, basophilic rim of neuroplasm and large vesicular nuclei (Figure 3).

In rats treated with 100 ppm sodium fluoride, the hippocampus revealed various degree of disarrangement of pyramidal layer (Figure 4). Many pyramidal neurons appeared distorted with elongated rod shaped nucleus (Figure 5) and were shrunken with reduced Nissl's granules (Figure 6) and contained darkly stained nucleus (Figure 7).

In rats treated with 200 ppm sodium fluoride, many neurons displayed irregularities in their structure and distribution in comparison to control group. There was decrease in the density of pyramidal neurons in CA1 region (Figure 8). In the polymorphic layer, neuropil displayed various degree of necrosis (Figure 9). In CA3 region, the pyramidal neurons were elongated with spindle shaped nucleus (Figure 10). In dentate gyrus region, granular cells showed vacuolation and disintegration of neuroplasm (Figure 11). The neuropil exhibited focal areas of necrosis (Figure 12). In rats treated with 300 ppm sodium fluoride,

neurotoxic changes were very much apparent compared to control group. There were present constricted blood capillaries in the neuropil (Figure 13) and nuclei of some nerve cells were pyknosed (Figure 14). The neuropil of polymorphic layer depicted degeneration and atrophied nerve cells (Figure 15). The pyramidal neurons appeared shrunken and darkly stained (Figure 16). The neuropil at some location appeared as clear halos (Figure 17). Some granule cells of dentate gyrus region (Figure 18) as well as pyramidal cell exhibited chromatolysis (Figure 19).

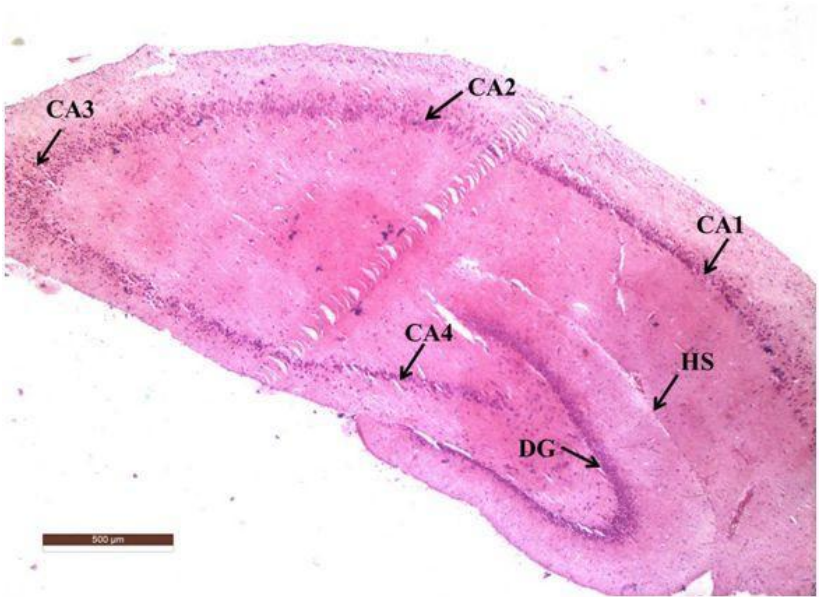

Figure 1: T. S of Hippocampus of Control Rat Showing Four Distinct Areas: CA1, CA2, CA3 and CA4; There is a Narrow Hippocampus Sulcus (HS) and Dentate Gyrus (DG). H&E, X40

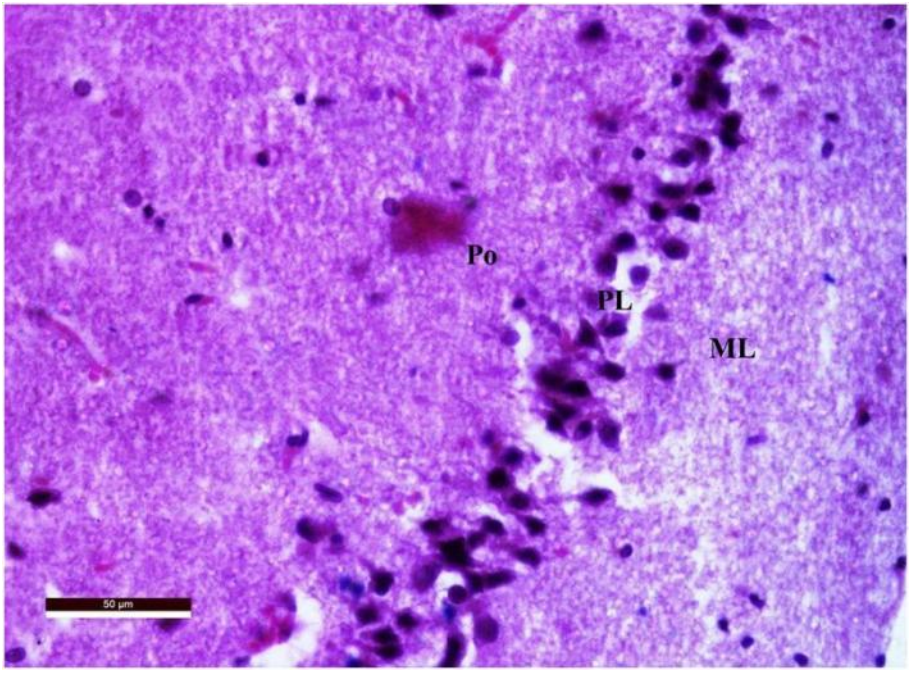

Figure 2: CA1 Area of Hippocampus of Control Rat Showing Molecular (M), Pyramidal (P), and Polymorphic (Po) Layers; Glial Cell and Capillaries are Scattered inside the Polymorphic Layer H&E, X400

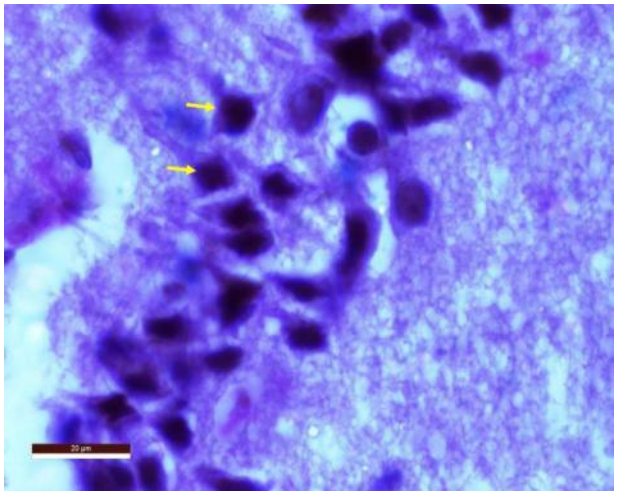

Figure 3: T. S of Hippocampus of Control Rat Showing Pyramidal Cell with Triangular Body, Nodded Axon Multiple Branched Dendrites with Spines, Basophilic Rim of Cytoplasm and Large Vesicular Nuclei H&E, X1000

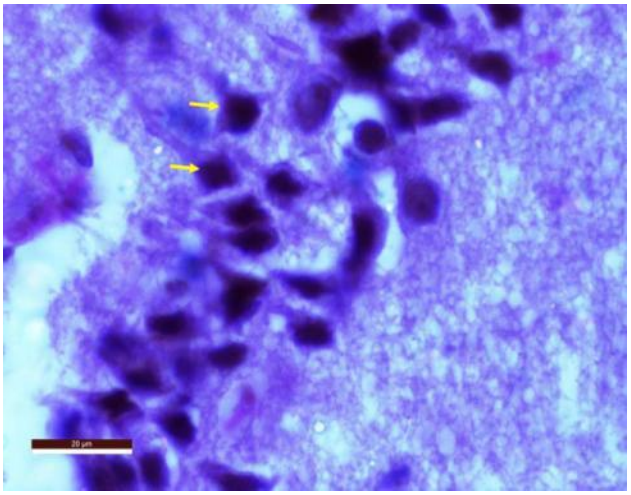

Figure 4: T. S of Hippocampus of Rat Treated with 100 ppm Sodium Fluoride Showing Disarrangement of Pyramidal Layer H&E, X400

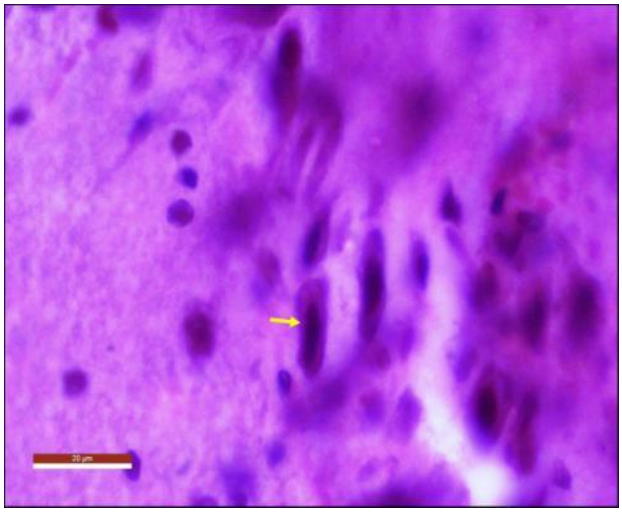

Figure 5: T. S of Hippocampus of Rat Treated with 100 ppm Sodium Fluoride Showing Distorted Pyramidal Cell with Elongated Rod Shaped Nucleus H&E, X1000

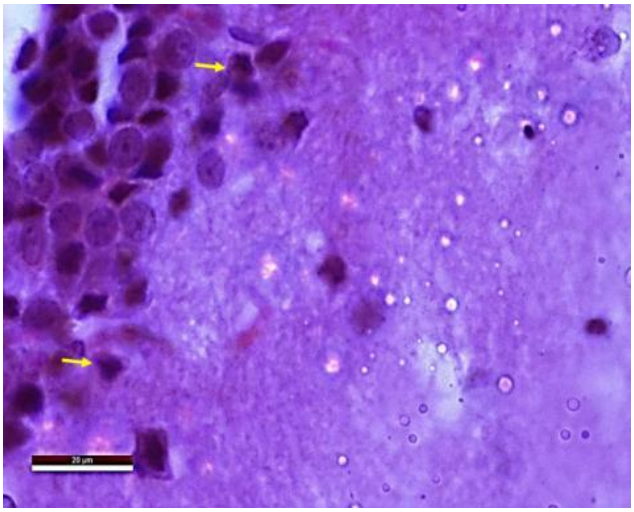

Figure 6: T. S of Hippocampus of Rat Treated with 100 ppm Sodium Fluoride Showing Shrinkage of Pyramidal Cells H&E, X1000

Discussion

The present study was undertaken to investigate the effect of different concentrations of sodium fluoride on neuropathological alterations in the hippocampus. Fluoride accumulated in the neurons and neuroglia showed morphological changes mainly in the hippocampus. Since fluoride is classified as neurotoxic substance, our neuropathological examination of the hippocampus confirmed that it is the most affected region due to fluoride intoxication. The neuropathological changes observed in our study in the form of necrosis, degeneration, atrophy, and pyknosis of pyramidal neuronsin Ammon's horn of hippocampus of the fluoride treated rat, is corroborated by earlier researcher (El-lethey *et al.,* 2010).

Neurodegenerative changes in glial cells, disarrangement of pyramidal layer, distortion in shape of pyramidal neurons, elongated rod shaped nucleus, shrunken and darkly stained nucleus, vacuolation and

disintegration in neurons and neuroglia, clear halos and chromatolysis were observed in various regions of hippocampus of rats treated with different doses of sodium fluoride. Earlier reports on effects of sodium fluoride on rat brain also documented loss of neuron cell bodies in hippocampus and loss of synaptic structure (Bhatnagar *et al.,* 2002; Shivarajashankara *et al.,* 2002; Zhang *et al.,* 2008), decline in number of neurons, presence of vacuolated cells and degenerated nerve cell bodies in the CA1 and CA3 areas of hippocampal sub-regions (Shashi and Sharma, 2015).

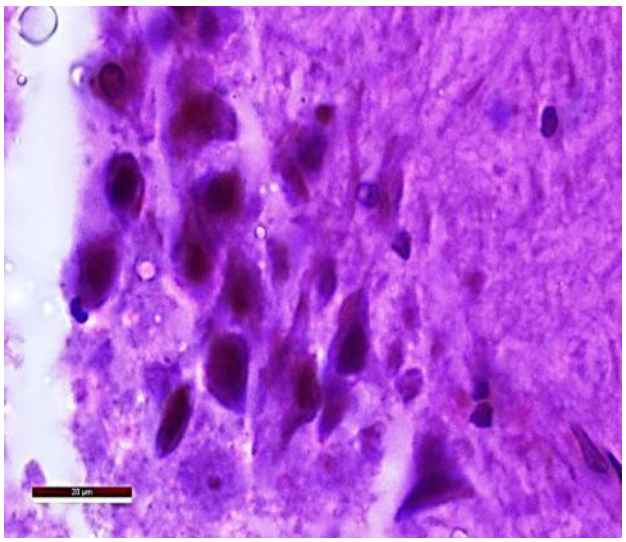

Figure 7: T. S of Hippocampus of Rat Treated with 100 ppm Sodium Fluoride Showing Darkly Stained Nucleus in Pyramidal Neurones H&E, X1000

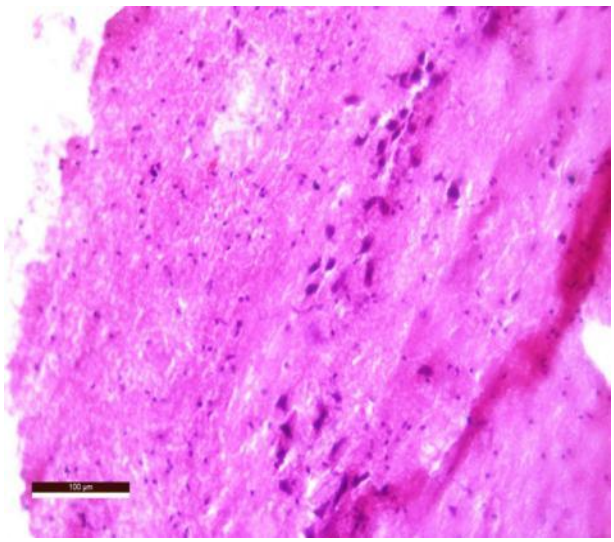

Figure 8: T. S of Hippocampus of Rat Treated with 200 ppm Sodium Fluoride Showing Decrease in the Density of Pyramidal Cells in CA1 Region H&E, X200

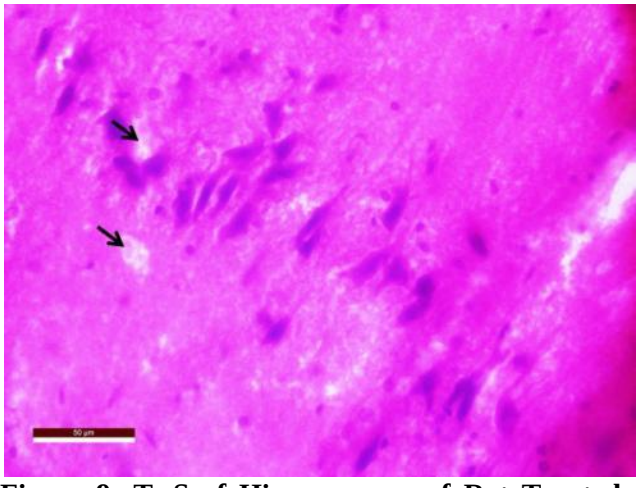

Figure 9: T. S of Hippocampus of Rat Treated with 200 ppm Sodium Fluoride Showing Necrosis of Neuropil H&E, X400

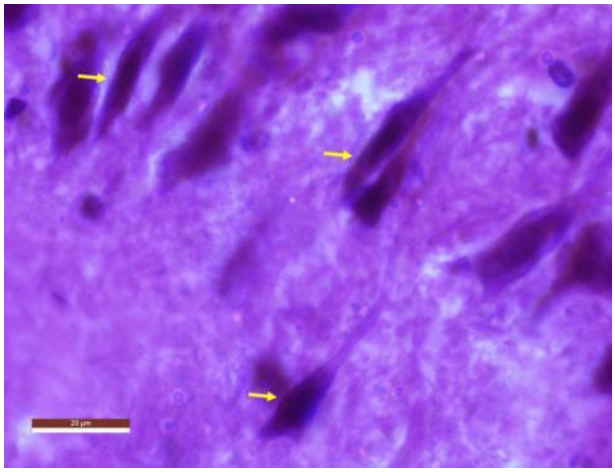

Figure 10: T. S of Hippocampus of Rat Treated with 200 ppm Sodium Fluoride Showing Elongated Pyramidal Cell with Spindle Shaped Nucleus H&E, X1000

Presence of degenerating neurons (Basha *et al.,* 2011), vacuolated swollen mitochondria and myelinated fibers with breaks in continuity (axon partly preserved and partly vacuolated) was observed in hippocampus of fluoride treated rats (Reddy *et al.,* 2011). The rats exposed to 1 ppm (50 µmol/L) of water fluoride for one year showed morphological alterations in the brain tissue (Varner *et al.,* 1998)

showed plausibility of our results. A decrease in the number of Purkinje cells, thickening and disappearance of dendrites, swelling of mitochondria, and dilation of endoplasmic reticulum in neurons (Guan *et al.,* 1998) along with impaired hippocampus synaptic interface structure (Zhang *et al.,* 2008) have been observed in the brains of experimental animals subjected to fluorosis. Fluoride treatment could lead to degeneration of glial cells.

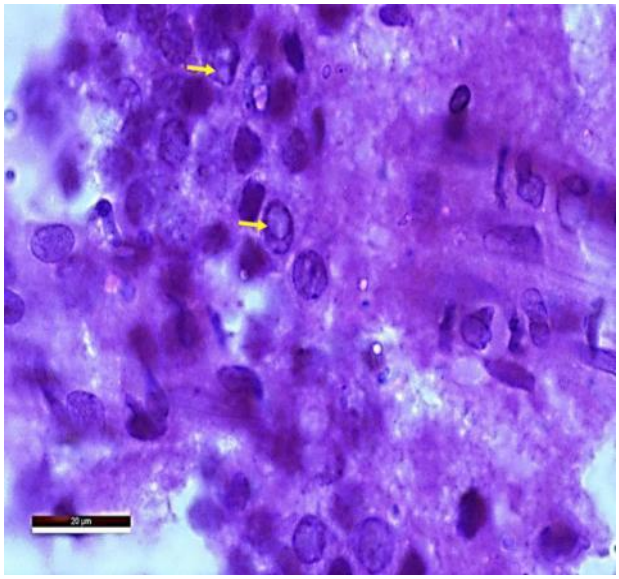

Figure 11: T. S of Hippocampus of Rat Treated with 200 ppm Sodium Fluoride Showing Vacuolation and Disintegration of Neuroplasm of Granule Cells H&E, X1000

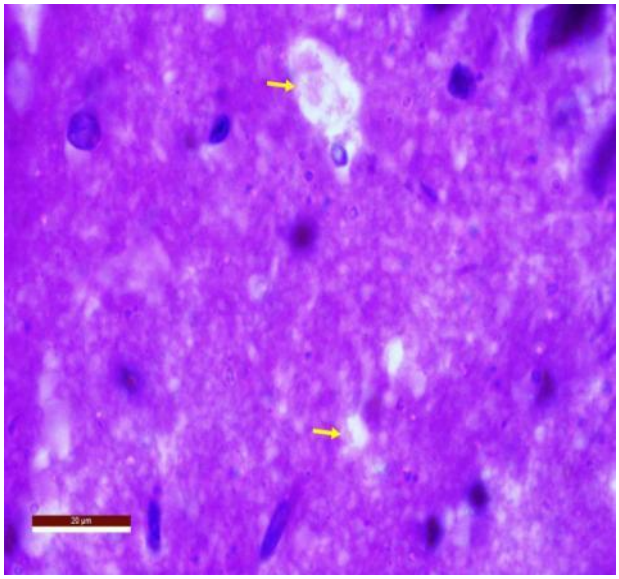

Figure 12: T. S of Hippocampus of Rat Treated with 200 ppm Sodium Fluoride Showing Focal Area of Necrosis in Neuropil H&E, X1000

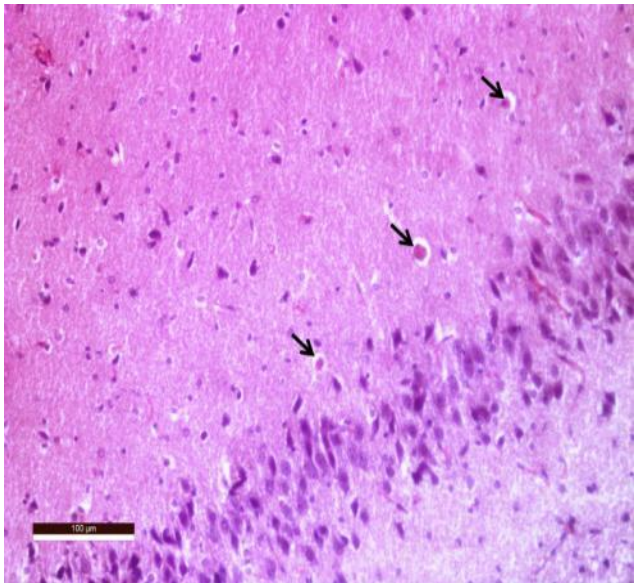

Figure 13: T. S of Hippocampus of Rat Treated with 300 ppm Sodium Fluoride Showing Constricted Blood Capillaries in the Neuropil H&E, X200

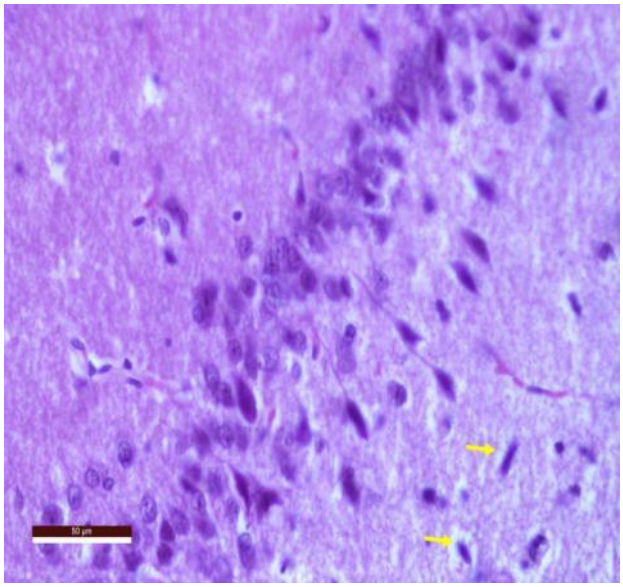

Figure 14: T. S of Hippocampus of Rat Treated with 300 ppm Sodium Fluoride Showing Pyknotic Nerve Cells H&E, X400

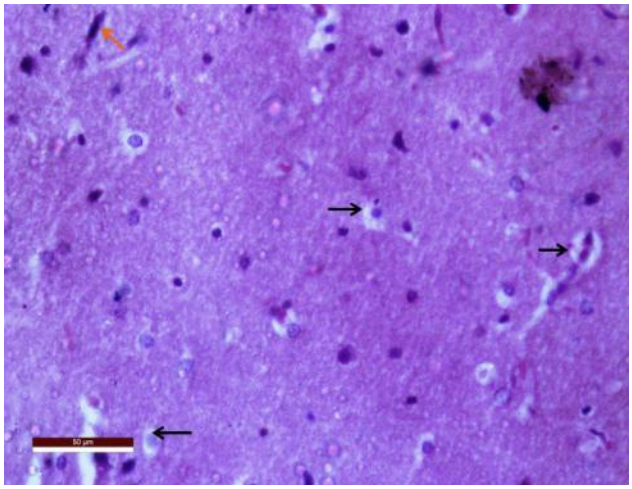

Figure 15: T. S of Hippocampus of Rat Treated with 300 ppm Sodium Fluoride Showing Degeneration of Neuropil (Black Colored Arrows) and Atrophied Nerve Cells (Orange Colored Arrow) H&E, X400

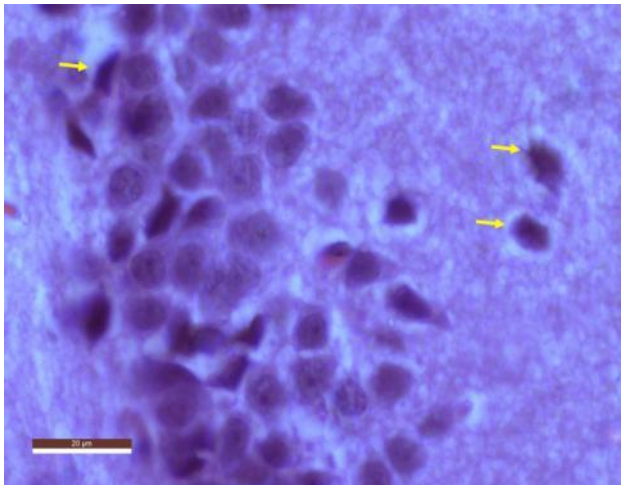

Figure 16: T. S of Hippocampus of Rat Treated with 300 ppm Sodium Fluoride Showing Dark Shrunken Pyramidal Cells H&E, X1000

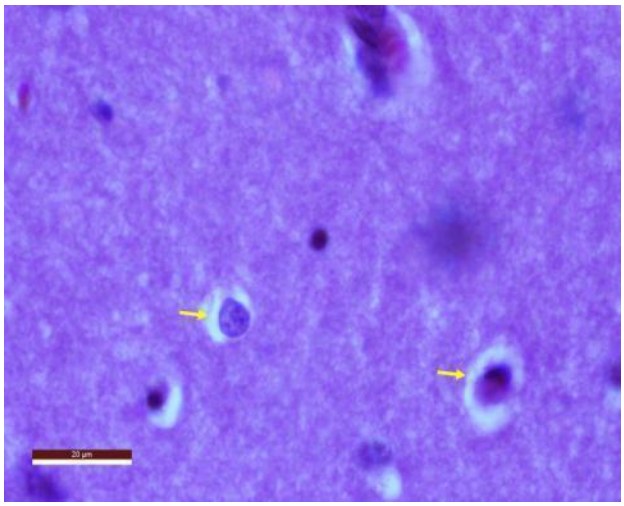

Figure 17: T. S of Hippocampus of Rat Treated with 300 ppm Sodium Fluoride Showing Clear Halos in the Neuropil H&E, X100

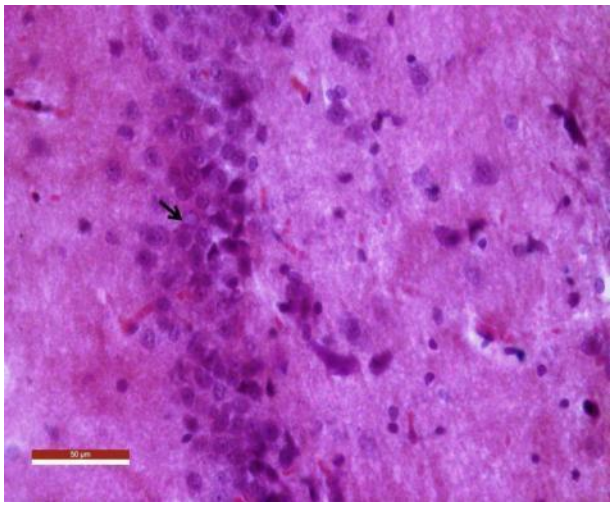

Figure 18: T. S of Hippocampus of Rat Treated with 300 ppm Sodium Fluoride Showing Chromatolysis in Granule Cells H&E, X400

Glial cells were as targets of fluoride toxicity and involved in dysfunction of the brain induced by fluoride (Trabelsi *et al.*, 2001). Sodium fluoride induced apoptosis and latter involved a series of biochemical events leading to characteristic changes in cell morphology and death. These changes include blebbing, loss of membrane symmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation and chromosomal DNA fragmentation (Kerr *et al.*, 1989). In some other study, reduced neuronal density in the CA3 region of hippocampus of rat due to fluorosis were documented (Nasir and Asad, 2013).

The most probable mechanism for the neurodegenerative effects of fluoride are likely related to excitotoxicity by free radicals, which impairs the glutamate removal by activating microglia which contain abundant stores of glutamate (Chirumari and Reddy, 2007; Blaylock, 2004; Pellegrini-Giampietro *et al.*, 1988).

The findings of present study concluded that fluoride may worsen the cytoarchitectural arrangement in the hippocampus by altering the structure of pyramidal neurons and neuroglia. The nucleus of pyramidal neurons in hippocampus may be the main organelle that gets affected and starts depicting variation in size and structure in different concentration of sodium fluoride.

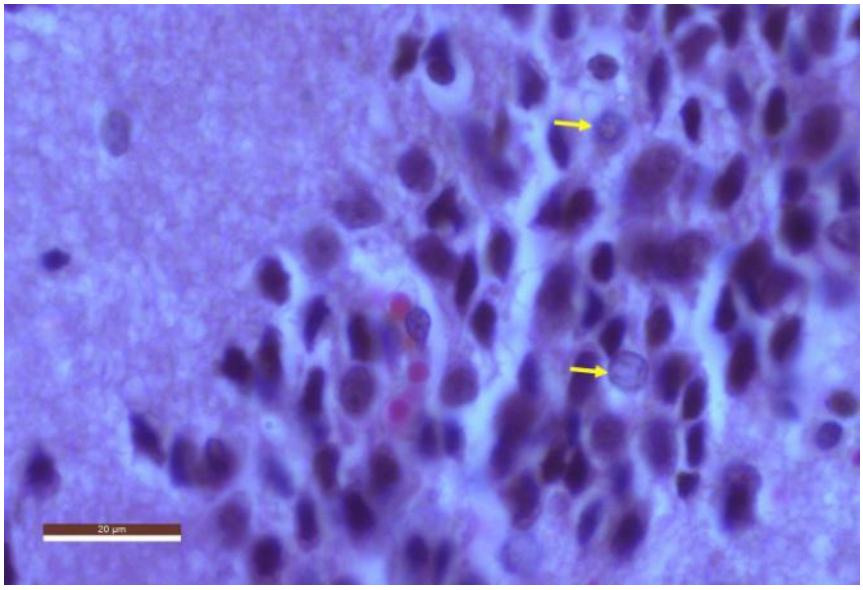

Figure 19: T. S of Hippocampus of Rat Treated with 300 ppm Sodium Fluoride Showing Chromatolysis in Neurones H&E, X1000

Conflict of Interest: The authors declare that there is no conflict of interest.

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REFERENCES
Basak A, Roy P and Sau S (2016). Evaluation of different clinico-radiological changes in endemic fluorosis and correlation with duration of exposure at Nasipur village of Birbhum district, West Bengal. *IOSR Journal of Dental and Medical Sciences* **5**(4) 94-107.
Basha MP and Sujitha NS (2011). Chronic Fluoride Toxicity and Myocardial Damage: Antioxidant Offered Protection in Second Generation Rats. *Toxicology International* **18**(2) 99-104.
Basha PM, Rai P and Begum S (2011). Fluoride toxicity and status of serum thyroid hormones,brain histopathology, and learning memory in rats: a multigenerational assessment. *Biological Trace Element Research* **144** 1083-1094.
Bhatnagar M, Rao P, Sushma J and Bhatnagar R (2002). Neurotoxicity of fluoride: neurodegeneration in hippocampus of female mice. *Indian Journal of Experimental Biology* **40**(5) 546-554.
Blaylock RL (2004). Excitotoxicity: a possible central mechanism in fluoride neurotoxicity. *Fluoride* **37**(4) 301-314.
Burgstahler AW and Colquhoun JC (1996). Neurotoxicity of fluoride. *Fluoride* **29** 57-58.
Chirumari K and Reddy P (2007). Dose-dependent effects of fluoride on neurochemical milieu in the hippocampus and neocortex of rat brain. *Fluoride* **40**(2) 101-110.
Drury RA and Wellington EA (1967). *Carleton's Histological Technique*, 4th edition, (Oxford University Press: New York, USA).

El-lethey HS, Kamel MM and Shaheed IB (2010). Neurobehavioral toxicity produced by sodium fluoride in drinking water of laboratory rats. *Journal of American Science* **6**(5) 54-63.
Guan Z, Wang Y, Xiao K, Dai D, Chen Y Liu *et al*., (1998). Influence of chronic fluorosis on membrane lipids in rat brain. *Neurotoxicology and Teratology* **20**(5) 537–542.
Hayman LA, Fuller GN, Cavazos JE, Pfleger MJ, Meyers CA and Jackson EF (1998). The hippocampus: normal anatomy and pathology. *American Journal of Roentgenology* **171**(4) 1139-1146.
Karaoz E, Oncu M, Gulle K, Kanter M, Gultekin F, Karaoz S *et al*., (2004). Effect of chronic fluorosis on lipid peroxidation and histology of kidney tissues in first- and second-generation rats. *Biological Trace Element Research* **102**(1-3) 199-208.
Kerr DS, Campbell LW, Hao SY and Landfield PW (1989). Corticosteroid modulation of hippocampal potentials: increased effect with aging. *Science* **245**(4925) 1505-1509.
Manna P, Sinha M and Sil PC (2007). A 43 kD protein isolated from the herb *Cajanusindicus* L attenuates sodium fluoride induced hepatic and renal disorders in vivo. *Journal of Biochemistry and Molecular Biology* **40** 382-395.
Nasir N and Asad MR (2013). Effects of flouride on CA3 region of hippocampus in adult albino rats. *Journal of Asian Scientific Research* **3**(7) 729-733.
Pellegrini-Giampietro D, Cherici G, Alesiani M, Carla V and Moroni F (1988). Excitatoryamino acid release from rat hippocampal slices as a consequence of free-radicalformation. *Journal of Neurochemistry* **51** 1960-1963.
Reddy PY, Reddy KP and Kumar KP (2011). Neurodegenerative changes in different regions of brain, spinal cord and sciatic nerve of rats treated with sodium fluoride. *Journal of Medical and Allied Sciences* **1**(1) 30-35.
Shashi A (2003). Histopathological investigation of fluoride induced neurotoxicity in rabbits. *Fluoride* **36** 95-105.
Shashi A and Kumar P (2016a). Toxicopathological and functional characterization of thyroid gland of rat in fluorosis. *International Journal of Basic and Applied Medical Sciences* **6**(1) 30-39.
Shashi A and Rana N (2016). Ultrastructural study of muscle fibers in experimental Fluorosis. *Journal of Basic and Applied Research* **2**(3) 266-271.
Shashi A and Sharma N (2015). Cerebral neurodegeneration in experimental fluorosis. *International Journal of Basic and Applied Medical Sciences* **5**(1) 146-151.
Shivarajashankara YM, Shivashankara AR, Bhat GP, Rao SM and Rao SH (2002). Histological changes in rat brain of young fluoride intoxicated rats. *Fluoride* **35** 12-21.
Spittle B (1994). Psycopharmacology of fluoride: a review. *International Clinical Psychopharmacology* **9** 79-82.
Trabelsi M, Guermazi F and Zeghal N (2001). Effect of fluoride on thyroid function and cerebellar development in mice. *Fluoride* **34** 165-173.
Varner JA, Jensen KF, Horvath W and Issaccson RL (1998). Chronic administration of aluminium fluoride or sodium fluoride to rats in drinking water: alterations in neuronal and cerebrovascular integrity. *Brain Research* **784**(1-2) 284-98.
Zhang Z, Xu X and Shen X (2008). Effect of fluoride exposure on synaptic structure of brain areas related to learning-memory in mice. *Fluoride* **41** 139-143.