ejpmr, 2018,5(7), 1-8

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

HISTOLOGICAL AND FUNCTIONAL EFFECT OF FLUORIDE ON CEREBRAL CORTEX OF THE BRAIN

Selemun Hagos[1], Girma Seyoum[2], Desta Gebeya[1] and Abraha Teklay[3]

[1]Adigrat University, College of Medicine and Health Science, Department of Biomedical Sciences, Human Anatomy Unit, Adigrat, Ethiopia.

[2]Addis Ababa University, College of Health Sciences, School of Medicine, Department of Anatomy.

[3]Adigrat University, College of Medicine and Health Sciences, Department of Biomedical Sciences, Medical Physiology Unit.

***Corresponding Author: Selemun Hagos**

Adigrat University, College of Medicine and Health Science, Department of Biomedical Sciences, Human Anatomy Unit, P.O. Box 50, Adigrat, Ethiopia.

Article Received on 22/04/2018 **Article Revised on 13/05/2018** **Article Accepted on 02/06/2018**

ABSTRACT

Fluoride is an ion that is derived from the highly electronegative fluorine gas. It is omnipresent in the environment and has been added to drinking water supplies with a recommended dose. Drinks, tooth pastes, mouth rinses, dietary supplements and foods are also considered as sources of fluorides. This paper reviews the scientific literatures linking fluoride with its effect on histology and function of cerebral cortex of the brain. The literatures were assessed by focusing on the dose of fluoride, duration of exposure, type of experimental animals used to measure the effect of fluoride. In this review the role of fluoride in region specific and sub-cellular distribution of the brain with the relation of its neurotoxicity is highlighted. The literatures reviewed in the present paper used mice, rats and rat offspring as experimental animals. From the literatures reviewed in the present paper, fluoride showed to be neurotoxic chemical which affects the biochemical content of brain, cause weight loss, cause neurodegeneration, histological alternation in hippocampus and cerebral cortex of the brain, disrupting behavioral activities and cause reduction in cognitive and memory functions.

KEYWORDS: Fluoride, Cerebral cortex, Histopathological effect, Weight loss, Biochemical change and Neurobehavioral toxicity.

INTRODUCTION

The fluoride ion is derived from the element fluorine, a gas that never occurs in a free state in nature. Fluoride is abundant in the environment and exists only in combination with other elements as fluoride compounds, which are constituents of minerals in rocks and soil. Therefore, fluoride is commonly associated with volcanic activity.[1]

Fluorine occurs in varying concentrations in rocks, soil, water, air, plants and animals both naturally and as a consequence of human activity such as agricultural or industrial processes.[2] The major sources of fluoride for humans include food containing fluoride and bony meals, as fluoride concentrates in bones of most mammals, dark green vegetables as the tea plant accumulate fluoride from soil and water, groundwater, fluoride supplements (such as fluoride tablets), fluoride dentifrices, and professionally applied fluoride gel, tooth pastes and mouth rinses.[1,2,3,4,5,6,7]

Fluoride compounds are used in ware and cable insulations, pipe linings, as rocket propellants, rodenticides, refrigerants, aerosol propellants, polymers for plastics, in the separation of uranium isotopes, and in the aluminum, beryllium, antimony, superphosphate fertilizer (which contain an average of 3.8% fluorine), glass, electronic ceramics, brick industries and sodium fluoride are used in municipal water fluoridation schemes.[3]

Higher exposure of fluoride causes different functional and histological health problems including: skeletal system, teeth, and brain and spinal cord and nerves.[8,9] Long term exposure of fluoride can cause denser bones, joint pain, and a limited range of joint movement.[7,10] In advanced stages of fluorosis, neurological manifestation such as paralysis of limbs, vertigo, spasticity in extremities, and impaired mental activity are observed in human beings.[11] Additionally, Fluoride intake causes significant dose-dependent reduction in the content of acidic, basic, neutral, and total protein content in the cerebral hemisphere, cerebellum and medulla oblongata.[12] It is now recognized that fluoride also affects cells from soft tissues, i.e., renal, endothelial, gonadal, and neurological cells.[13]

Effect of fluoride on body weight

Effects of fluoride on body weight have a direct relationship with the strength of dose and length of time of exposure. Exposure to different concentrations of fluoride for different durations induced an average body weight gain significantly lower in group of rats exposed to high fluride doses for long duration, with distinct hair loss.[14]

In the study conducted by Hamid *et al.*[15] who showed that administration of fluoride at a dose level of 10, 100 and 500 ppm as NaF in drinking water for 3 months induced animals of the experimental groups looked weaker. Moreover, the animals which received highest amount of fluoride in their drinking water for long period of time were evidenced by definite loss of body weight. As the time of exposure increases the animals treated with fluoride were also looked more lethargic, particularly after 60 and 90 days of fluoride administration (Figure 1).

Administration of fluoride at dose level of 0, 1, 5, 10, and 20 mg NaF/kg bw/day intraperitoneally for 14 days induced significantly decreased body weight and brain index as fluoride levels increased in the hippocampus and neocortex.[16] Similar findings also conducted by Trabelsi *et al.*[17] on mice reported that the 14-day-old mice whose mothers had been treated with NaF, had a 35% decrease in body weight compared with the control group but no significant change in the weight of cerebellar or cerebral cortex. The weight loss might be due to inhibition by fluorides of protein synthesis and/or breakdown of protein.[18] It could also be attributed to the toxic effect of fluorides on the tissues and the metabolism of the body.[15]

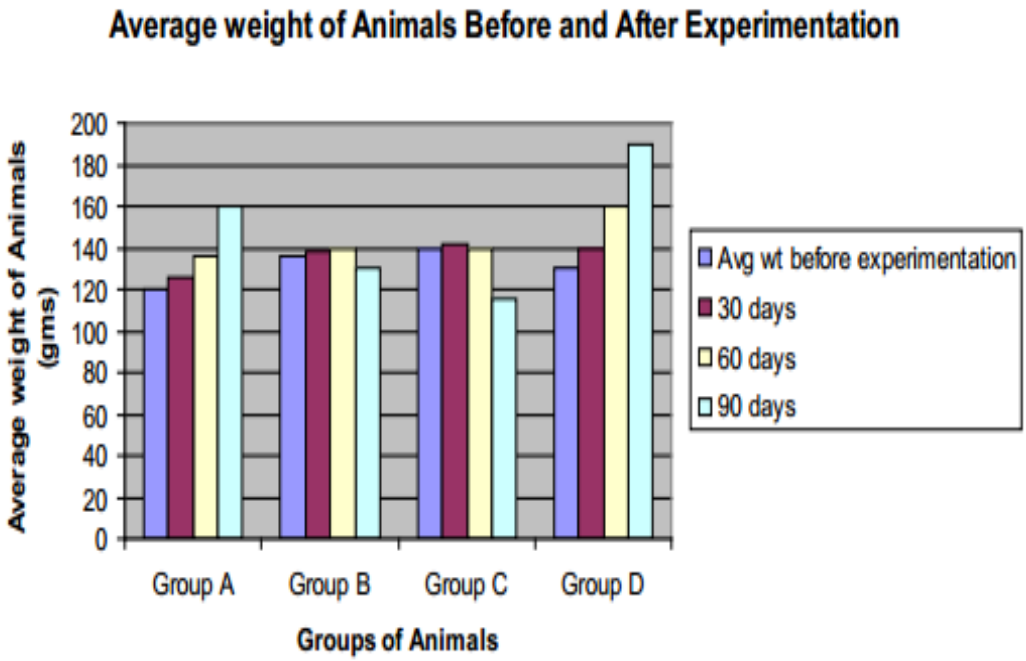

Figure 1. Average weight of animale before and after experimentation adopted from Hamid *et al.*[15]

Neurodegenerative and Histopatological Changes

Fluoride can pass through the blood-brain barrier, and fluoride accumulated in brain tissue might interfere with the metabolism of brain phospholipids, which is related with the degeneration of neurons. The changes in brain phospholipid metabolism could be involved in the pathogenesis of chronic fluorosis.[19] Brain plays a key role in memory, attention, perceptual awareness, thought, language, and consciousness. It also integrates higher mental functions, general movement, visceral functions, and behavioral reactions.[20] Fluoride exposure also has neurodegenerative effects on the histology of cerebral cortex which is evidenced by learning and memory deficits and impairment of motor activities as well as in behavioral alternation.[14,21]

Histopathological examination of fluoride intoxicated hippocampus at different doses for different duration's revealed distinct neurodegenerative changes of nerve cells.[14] Poisoning of rats to fluoride induce reduced neuronal density more pronounced in the CA3 region of hippocampus.[21] Moreover, examination on light microscopy of the hippocampus, showed reduced neuronal density more pronounced in the CA3 region of hippocampus.[22]

An exposure to high doses of fluoride induces significant neurodegenerative changes in the motor cortex. These changes included decrease in size and number of neurons in all the regions, signs of chromatolysis and gliosis in the motor cortex. These histological changes suggest a toxic effect of high dose fluoride intake & on chronic use (Fig 2).[15]

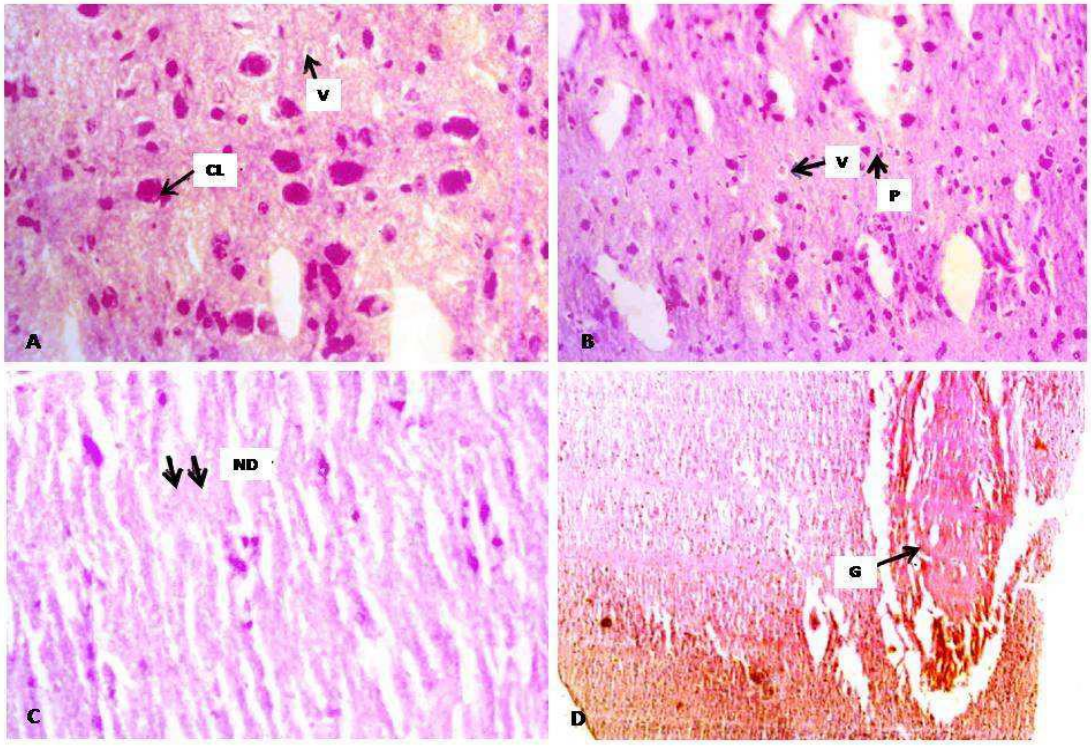

Figure 2. Histological changes in cerebrum adopted from Hamid *et al*. [15]

- A= Arrow heads showing neuronal swelling, signs of Chromatolysis, Vacoulation
- B= Arrow heads showing Pyknotic change
- C= Arrow heads showing decrease in Neuronal Density
- D= Arrow heads showing Gliosis

The study conducted by Shashi[23] also reported neuropathological changes occurred with loss of the molecular layer and glial cell layer in the brain tissues of rabbits exposed to the three higher fluoride doses. The toxic effect of fluoride is evidenced by chromatolysis and reduced Purkinje cells, gliosis, central nervous system problems such as tremors, seizures, and paralysis indicating brain dysfunction seen at the highest doses.[14,19,23,24,25]

Other study conducted by Shivaraiashankara *et al*.[11] also demonstrated that exposure of rats to high dose of fluoride (as NaF) in drinking water during their fetal, weanling, and post-weaning stages until the age of ten weeks induced significant neurodegenerative changes in the hippocampus, amygdala, motor cortex, and cerebellum. Changes included decrease in size and number of neurons in all the regions, decrease in the number of Purkinje cells in the cerebellum, and signs of chromatolysis and gliosis in the motor cortex. These histological changes suggest a toxic effect of high-fluoride intake during the early developing stages of life on the growth, differentiation, and subcellular organization of brain cells in rats.

Fluoride can also cause shrunken, pyknotic, and darkly stained with small nuclei of neurons, and decrease overall cell number.[11,8,26] Neurodegenerative and neurotoxic changes such as vacuolated in hippocampus and degenerated cell bodies were also noted due to exposure to fluoride.[40] Pyramidal cells and nerve cells of cerebral cortex revealed chromatolysis, edema, atrophy, necrosis and neuronophagia in different regions of brain (neocortex, hippocampus) as time of exposure and level of the dose increases.[23] This is certain that fluoride induces neurotoxicity treatment groups when compared with the control.[26,27]

The most probable mechanism for the neurodegenerative effects of fluoride are likely related to excitotoxicity by free radicals and lipid peroxidation which impairs the glutamate removal and by activating microglia which contain abundant stores of glutamate.[8,16,27] Lipid peroxidation and apoptosis may co-exist at the beginning when human tissues are exposed to excessive fluoride, and lipid peroxidation generates a lot of free radicals that may be sufficient to cause apoptosis.[28] Continuous exposure of NaF increases nitric oxide synthase activity which plays a major role in all neurodegenerative

diseases, primarily by damaging mitochondrial energy production, inhibiting glutamate reuptake and stimulating lipid.[29]

Histological alterations were seen in cornu ammonis-1 (CA1), cormu ammonis-4 (CA4), and dentate gyrus sub regions of the hippocampus.[11] Reduced neuronal density on CA3 region of the hippocampus was also registered as a result of exposed to fluoride intoxication for 7 weeks with 20ppm of NaF.[21]

Similarly, application of 20ppm of NaF on rats for 4 weeks brings harmful effects for the nervous tissue in hippocampus and causes non reversible neuronal damage leading to loss of neurons.[9] In addition, fluoride intoxication causes pyramidal cell neuronal degeneration in all regions of ammons horn (CA1-CA4) and granule cells in dentate gyrus, though the degeneration was more pronounced in CA3.[30]

Neurotoxicity and Neurobehavioral Effects of Fluoride

In an attempt to model human neurobehavioral changes in rodents, a wide range of behavioral testing paradigms have been developed. Many of these tests induce reduced memory retention like maze test for learning and memory, novelty acquisition test, Open field revealed and general motor activity.

An experimental study conducted by Saad El-Dien *et al*.[14] to assess the effect of fluoride associated with behavioral and brain modifications showed that; administration of fluoride in the form of Na-F in drinking water at one of three concentrations; 0, 50 and 100 ppm from second trimester of pregnancy till weaning of their pups at 30 days of age resulted in behavioral and morphological alternation of the brain such as impairment of exploratory motor activities (EMA) and marked impairment in habituation. An increased MDA level which indicates mild brain damage induced by higher concentration of NaF exposure was also reported in rats exposed to higher fluoride concentration.[41] Besides, learning and memory assessed during maze test and smaller number of platform crossings showed decreased spatial learning and memory retention in rats exposed to higher concentration of NaF in their drinking water for long periods.[14,41]

Similarly, higher sodium fluoride intake causes impairment in the open-field habituation and two-way active avoidance responses.[31] In addition, Fluoride poisoned Sprague- Dawley rats tested on at 120mg/L NaF for 3 months in water maze test induce significant decrease of learning and memory ability.[32] An experiment conducted in children between 6-11 year olds in makoo (Iran) also confirmed that children who used to drink high fluoride water showed significantly lower average IQ compared to children living with standard water fluoride level.[33]

Several studies across the world have reported the effects of fluoride in drinking water on cognitive capacities.[34,35] Among the studies, the one conducted by Xiang *et al.*[35] on 512 children (ages 8-13) living in two villages of china (Wamiao and Xinhuai) with different fluoride concentrations in drinking water to assess the IQ. The IQ test was administered in a double-blind manner. The results of the study showed that, the high-fluoride area (Wamiao) had a mean water concentration of 2.47 ± 0.79 mg/L (range 0.57-4.50 mg/L), and the low-fluoride area (Xinhuai) had a mean water concentration of 0.36 ±0.15 mg/L (range 0.18-0.76 mg/L). Thus, the average intelligence quotient (IQ) of the children in Wamiao was found to be significantly lower (92.2 ± 13.00; range, 54-126) than that in Xinhuai (100.41 ±13.21; range, 60-128).[34,35]

A study conducted by Lu et al.[34] in a different area of China also compared the IQs of 118 children (ages 10-12) living in two areas with different fluoride concentrations in the water (3.15 ± 0.61 mg/L in one area and 0.37 ± 0.04 mg/L in the other). The result showed significantly lower mean IQ scores among children in the high-fluoride area (92.27 ± 20.45) than in children in the low-fluoride area (103.05 ± 13.86). Of special importance, 21.6% of the children in the high-fluoride village scored 70 or below on the IQ scale. For the children in the low-fluoride village, only 3.4% had such low scores. In addition, urinary fluoride concentrations were inversely correlated with mental performance in the IQ test. Another scholar suggested that, an increasing fluoride level in drinking water greatly reduces IQ, intelligence, learning, and memory in children and increases mental retardation.[36,41]

Administration of fluoride may cause motor impairment. Sodium fluoride treated experimental animals (rats) at dose level of 20 ppm for 7 weeks became sluggish/ less reactive.[21] NaF induces sever neurohistopathological changes in the brain which leds to paralysis of limbs.[23] Moreover, exposure of animals to 20 mg NaF showed hemiplegia and animals administered 50 mg of NaF/Kg bw/day, spastic paraplegia, quadriplegia, tremors, and seizures were recorded.[23]

Neurochemical and Biochemical Changes

Several experimental animal studies have examined biochemical changes in the brain associated with fluoride. Fluoride may induce production of free radicals. Studies of brains on laboratory animals indicates that, continuous exposure of fluoride may induce significant reduced amount of enzymes and biochemical molecules such as lipids and phospholipids, phosphohydrolases and phospholipase D and inhibit the activity of cholinesterases that are relevant for mental stability and adequate recovery of memories as well as increase the risk of developing Alzheimer's disease.[13,25,39]

Oral administration of 12 mg/kg of NaF caused significant dose-dependent reduction in the content of

acidic, basic, neutral, and total protein content in the cerebral hemisphere cerebellum and medulla oblongata regions of mice brain.[12] Fluorides affect the concentration of neurotransmitter and their functions which involves in the regulation of glucagons, prostaglandins, and a number of central nervous system peptides, including vasopressin, endogenous opioids, and other hypothalamic peptides.[13,39] Fluorides given to experimental animals as NaF or AlF evidenced by changed histological appearance of cerebral hemisphere such as distortion in cells in the outer and inner layers of the neocortex, pronounced neuronal deformations in the hippocampus, limited extent of deformations in amygdale, the cerebellum, signs of dementia and decreased levels of DNA and RNA.[13,39] Besides AlF provides false messages throughout the nervous system and diminishes the energy essential to brain function at the same time.[13]

The study conducted by Chirumari and Reddy[16] revealed decreased levels of antioxidants in the hippocampus and neocortex as a response to high fluoride intake. The decrease in antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione transferase (GST), accompanied with an increase in the pro-oxidative markers lipid peroxidation (LPO) and xanthine oxidase (XOD) in the NaF treated witar rats is suggestive of oxyradical release (Table 1). Several experimental studies in mice across the world approved the functional impairment of SOD, GST, and catalase enzymes as a result of fluoride exposure.[28,38]

Chirumari and Reddy[16] also added high exposure of rats to fluoride induce significant increase in the levels of dopamine, serotonin, 5-hydroxyindoleacetic acid and homovanillic acid in the hippocampus and neocortex of the NaF treated rats compared with the controls. On the other hand, norepinephrine and epinephrine levels were decreased significantly in the hippocampus and neocortex of the NaF treated rats compared with the controls. In addition, the protein kinase C (PKC) activity increased significantly in the hippocampus as well as the neocortex (Table 2).

Table 1: Free radical enzymes in hippocampus and neocortex of brain in control and NaF treated rats Chirumari and Reddy.[16]

Neocortex					
Free radical enzymes Mean±SD	**Group I (Control)**	**Group II**	**Group III**	**Group IV**	**Group V**
Catalase	5.22±0.10	4.73±0.08	4.12±0.10	3.42±0.08	2.88±0.10
GPX	4.06±0.13	4.92±0.10	5.29±0.10	5.72±0.10	6.11±0.13
GST	8.33±0.09	7.69±0.11	7.17±0.10	6.83±0.09	6.10±0.12
LPO(MDA)	4.16±0.11	4.79±0.11	5.18±0.12	5.73±0.09	6.03±0.15
SOD	5.79±0.11	5.18±0.11	4.73±0.10	3.76±0.13	3.30±0.12
XOD	1.43±0.09	2.04±0.14	2.31±0.08	2.82±0.11	3.13±0.11

Hippocampus					
Free radical enzymes Mean±SD	**Group I (Control)**	**Group II**	**Group III**	**Group IV**	**Group V**
Catalase	3.73±0.09	3.12±0.12	2.74±0.11	2.17±0.10	1.73±0.12
GPX	2.47±0.42	2.81±0.09	3.23±0.10	3.85±0.12	4.29±0.10
GST	6.73±0.10	5.80±0.10	5.22±0.10	4.83±0.09	4.33±0.09
LPO(MDA)	2.26±0.11	2.85±0.11	3.10±0.12	3.32±0.09	3.69±0.10
SOD	3.37±0.09	2.81±0.09	2.25±0.11	1.80±0.12	0.91±0.10
XOD	0.21±0.08	0.78±0.11	1.05±0.13	1.33±0.09	1.73±0.09

The enzyme activities expressed as: Lipid peroxidation (LPO) (MDA-malondialdehyde) (micromoles of MDA/gm wet wt of tissue), xanthine oxidase (XOD) (micromole of formazan formed/mg protein/hr), superoxide dismutase (SOD) (Units/mg protein/minute), Catalase (Units/mg protein/minute), glutathione-s-transferase (GST) (micromoles of thioether formed/mg protein/min), glutathione peroxidase (GPX) (micromoles of NADPH oxidized/mg protein/min). Values are Mean±SD of six animals per group. Statistical analysis was carried out using one way ANOVA followed by Tukey's multiple comparison test and significance set at $p<0.05$. The values of multiple comparison test were significant ($p<0.05$) among group I, II, III, IV and V.

Table 2: Neurotransmitter levels in hippocampus and neocortex of brain in control and NaF treated rats with variable doses of NaF Chirumari and Reddy.[16]

Neocortex					
Neurotransmitters Mean±SD	**Group I (Control)**	**Group II**	**Group III**	**Group IV**	**Group V**
Dopamine	0.52±0.08	1.10±0.10	1.72±0.10	2.17±0.09	2.90±0.11
NE	0.94±0.08	0.68±0.08	0.52±0.09	0.38±0.09	0.11±0.07
Epinephrine	2.59±0.12	2.23±0.08	1.93±0.08	1.53±0.09	0.82±0.09
5-HIAA	2.32±0.11	2.82±0.10	3.22±0.08	3.69±0.11	4.05±0.12
HVA	0.31±0.10	1.31±0.09	1.74±0.11	2.18±0.11	2.84±0.13
Serotonin	1.20±0.12	1.82±0.09	2.26±0.08	2.78±0.11	3.31±0.08

Hippocampus					
Neurotransmitters Mean±SD	**Group I (Control)**	**Group II**	**Group III**	**Group IV**	**Group V**
Dopamine	0.12±0.09	0.78±0.10	1.11±0.09	1.53±0.08	1.89±0.12
NE	1.91±0.10	1.57±0.07	1.07±0.09	0.62±0.09	0.25±0.07
Epinephrine	1.92±0.10	1.62±0.08	1.35±0.10	0.68±0.38	0.49±0.10
5-HIAA	1.48±0.08	1.91±0.09	2.24±0.11	2.74±0.10	2.97±0.10
HVA	0.16±0.10	0.42±0.08	0.92±0.08	1.11±0.10	1.29±0.10
Serotonin	0.33±0.08	0.92±0.09	1.25±0.11	1.81±0.11	2.06±0.12

All the neurotransmitter levels (dopamine, norepinephrine (NE), epinephrine, 5-hydroxy-indoleacetic acid (5-HIAA), homovanillic acid (HVA), and serotonin) are expressed as microgram per gram wet weight. Values are Mean±SD of six animals per group. Statistical analysis was carried out using one way ANOVA followed by Tukey's multiple comparison test and significance set at $p<0.05$. The values of multiple comparison test were significant ($p<0.05$) among group I, II, III, IV and V.

Similar findings were also reported by Bhatnagar[37] and Shah and Chinoy[39] who demonstrated that the adverse effects of fluoride on the cerebral cortex of mice revealed that the activity of acetylcholinesterase in brain and the DNA and RNA levels in the cerebral cortex decreased significantly as compared to control. The AChE activity was also significantly decreased in brain of fluoride treated mice.[38] Thus the decrease in AChE may interfere with the synaptic transmission in brain and muscle (Table 3). About 10ppm NaF can induce significant toxicity and induce changes in ACh content and AChE activity. This fluoride intoxication markedly affects cholinergic system, which may result in dysfunctions of neurotransmission in brain, and the fluoride induced neurotoxicity.[24,41]

Table 3: Effects of F in drinking water on various parameters in the hippocampus of mice Bhatnagar *et al.*[37]

Group	**Control**	**60 ppm (E1)**	**120 ppm (E2)**
AchE (ΔOD)	0.11± 0.01	0.062±0.01	0.022±0.004§
BchE (ΔOD)	0.09± 0.009	0.027±0.011‡	0.017±0.023
SOD (% inhibition of NBT reduction)	56.55± 1.30	49.99±0.91§	38.10±0.862§
CAT (µmoles H2O2utilized/min/mg of protein)	53.57± 6.26	27.38±4.06§	10.71±2.27§
MDA (nmoles/mL)	1.85± 0.32	1.21±0.28	4.67±0.68‡
Ascorbic acid (mg/100mL)	0.87± 0.20	1.83±0.17	2.24±0.22‡
Total protein (mg/mL)	0.10± 0.00	0.09±0.00	0.032±0.005§

Data represented as mean ±S.E.*P<0.05; P<0.02; P<0.01; P<0.001; no sign = non significant.

CONCLUSION

Scientific literatures reviewed in this paper revealed that fluoride affects the functions and histology of cerebral cortex of brain in various mechanisms in different species of animal model (mice, rats and neonatal rats). Studies in the animal model provided evidence of damage to the brain histology and function in different mechanism from exposure to fluoride. Results of these experimental studies showed that the brains of these animals were susceptible to fluoride toxicity. Various scientific findings reviewed in this paper also reported that fluoride causes extensive damage to the brain by accelerating oxidative damage to biomolecules like lipid, protein and nucleic acids. Studies also reported that exposure to fluoride produced accumulated flouride levels in the brain as well as cognitive deficits and other changes, including decreased maze-learning ability, altered general motor activity, and histopathological alternation in the brain.

Conflict of interest- no.

Acknowledgement- not applicable.

REFERENCES

1. Barbiera O., Mendoza L.A. and Del Razo L. M. (2010). Molecular Mechanisms of Fluoride Toxicity. Chemico-Biological Interactions, 188: 319-333.
2. WHO (2002). Fluorides. Geneva, Switzerland: World Health Organization. Environmental Health Criteria Number, 227.
3. Pratusha G., Banji F., Bangi D., Ragina M. and Pavani B. (2011). Fluoride Toxicity- A Harsh Reality. IRJP., 2(4): 79-85.
4. US EPA (2010). Fluoride: Exposure and Relative Source Contribution Analysis. Washington, DC, U.S. Environmental Protection Agency. Health and Ecological Criteria Division Office of Water.
5. Tinanoff N. and O'Sullivan D.M. (1997): Early childhood caries: Overview and recent findings. Pediatr. Dent., Jan-Feb. 19(1): 12-16.
6. Edmunds W.M. and Smedley P.L. (1996). Groundwater geochemistry and health: an overview, in: Appleton, Fuge, McCall (Eds.), Environmental Geochemistry and Health, Geological Society Special Publication, 113: 91–105.
7. ASTDR (2003). Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine. U.S. Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry.
8. Basha M.P., Begum S. and Madhusudhan N. (2014). Antioxidants in the Management of Fluoride Induced Neural Oxidative Stress in Developing Rats. IJPSR., 5(1): 201-206.
9. Reddy V.B., Monika T. and Sasikala P. (2013). Study on Flouride Effect on Hippocampus in Adult Swiss Albino Rats. International Journal of Advanced Scientific and Technical Research, 4: 303-308.
10. Reddy D. (2009): Neurology of endemic skeletal fluorosis. Neurol India, 57(1): 7-12.
11. Shivaraiashankara Y.M., Shivashankara A.R., Gopalakrishna P. B., Muddanna S. R. and Hanumanth S. R. (2002). Histological Changes in the Brain of Young Fluoride. Intoxicated Rats. Fluoride, 35: 12-21.
12. Trivedi M.H., Verma R.J. and Chinoy N.J. (2007). Amelioration by Black Tea of Sodium Fluoride-Induced Changes in Protein Content of Cerebral Hemisphere, Cerebellum and Medulla Oblongata in Brain Region of Mice. Acta Poloniae Pharmaceutica - Drug Research, 64(3): 221-225.
13. National Research Council (NRC) (2006). Fluoride in drinking-water; A scientific review of EPA's standards, Washington DC, 2006.
14. Saad El-Dien H M., El Gamal D., Mubarak H. and Saleh S. (2010). Effect of Fluoride on Rat Cerebellar Cortex: Light and Electron Microscopic Studies. Egypt. J. Histol., 33(2): 245 – 256.
15. Hamid S., Kawoosa Z., Hamid S., MIR M.A., Hafiz A., Jan I. and Yaqoob F. (2012).
16. Chirumari K. and Reddy P.K. (2007). Dose-Dependent Effects of Fluoride on Neurochemical Milieu in the Hippocampus and Neocortex of Rat Brain. Fluoride, 40(2): 101–110.
17. Trabelsi M., Guermazi F. and Zeghal N. (2001). Effect of Fluoride on Thyroid Function and Cerebellar Development in Mice. Fluoride, 34(3): 165-173.
18. Trivedi M.H., Verma R.J., Sangai N.P. and Chinoy N.J. (2012). Mitigation by Black Tea Extract of Sodium Fluoride-Induced Histopathological Changes in Brain of Mice. Fluoride, 45(1): 13–26.
19. Du L., Wan C., Cao X. and Liu J. (2008). The Effect of Fluorine on the Developing Human Brain. Fluoride, 41(4): 327–330.
20. Brodal, S. (1977). The Central Nervous system: Structure and Function. Oxford University Press.
21. Nasir N. and Asad R. (2013). Effects of Flouride on Ca3 Region of Hippocampus in Adult Albino Rats. Journal of Asian Scientific Research, 3(7): 729-733.
22. Reddy M., Monika T. and Sasikala P. (2013). Study on Flouride Effect on Hippocampus in Adult Swiss Albino Rats. International Journal of Advanced Scientific and Technical Research, 4: 303-308.
23. Shashi A. (2003). Histopathological Investigation of Fluoride-Induced Neurotoxicity in Rabbits. Fluoride, 36(2): 95-105.
24. Sandeep V., Kavitha N., Praveena M., Ravi Sekhar P. and Jayantha Rao K. (2013). Effect of NaF on Albino Female Mice with Special Reference to Behavioral Studies and ACh and AChE Levels. Int. J. of Pharm. & Life Sci., 4: 2751-2755.
25. Sumathi T., Shobana C., Mahalakshmi V., Sureka R., Subathra M., Vishali A. and, Rekha K. (2013). Oxidative Stress in Brains of Male Rats Intoxicated with Aluminium and Neuromodulating Effect of *Celastrus Paniculatus* Alcoholic Seed Extract. *Asian J Pharm Clin Res, 6: 80-90.*
26. Ge Y., Ning H., Wang S. and Wang J. (2005). Effects of High Fluoride and Low Iodine on Brain Histopathology in Offspring Rats. Fluoride, 38(2): 127–132.
27. Akinrinade I. D. , Ogundele, O. M., Memudu A. E., Adefule A. K. and Kalejaiye E. D. (2013). Degeneration of Neuronal Cells: A Product of Fluoride and Aluminium Assault to the Prefrontal Cortex. Journal of Cell and Animal Biology, 7(6): 63-66.
28. Wang A., Xia T., Chu L., Zhang M., Liu F., Chen X. and Yang K. (2004). Effects of Fluoride on Lipid Peroxidation, DNA Damage and Apoptosis in Human Embryo Hepatocytes. Biomedical and Environmental Sciences, 17: 217-222.
29. Chauhun S., Ojha S. and Mahmood A. (2013). Effect of Fluoride and Ethanol Administration on Lipid Peroxidation Systems in Rat Brain. Indian Journal of Experimental Biology, 51: 249-255.
30. Bhatnagar M., Rai P. and Jain S. (2002). Neurotoxicity of fluoride: neurodegeneration in hippocampus of female mice. Indian journal of experimental biology, 40: 546-554.

31. Chioca L., Raupp I., Da Cunha C., Losso E. and Andreatini R. (2008): Subchronic fluoride intake induces impairment in habituation and active avoidance tasks in rats. Eur J Pharmacol, 579(1-3): 196-201.
32. Jiang S., Su J., Yao S., Zhang Y., Cao F., Wang F., Li J. and Xi S. (2014). Fluoride and Arsenic Exposure Impairs Learning and Memory and Decreases mGluR5 Expression in the Hippocampus and Cortex in Rats. Plose., 9(4): 1-10.
33. Seraj B., Shahrabi M., Shadfar M., Ahmadi R., Fallahzadeh M., Farrokh Eslamlu H. and Kharazifard M.J. (2012). Effect of High Water Fluoride Concentration on the Intellectual Development of Children in Makoo/Iran. Journal of Dentistry, Tehran University of Medical Sciences, 9(3): 221-229.
34. Lu Y., Sun Z.R., Wu L.N., Wang X., Lu W. and Liu S.S. (2000). Effect of high-fluoride water on intelligence in children. Fluoride, 33(2): 74-78.
35. Xiang Q., Liang Y., Chen L., Wang C., Chen B., Chen X. and Zhou M. (2003). Effect Of Fluoride In Drinking Water On Children's Intelligence. Fluoride, 36(2): 84-94.
36. Xiang Q., Liang Y., Chen B. and Chen L. (2011). Analysis of Children's Serum Fluoride Levels in Relation to Intelligence Scores in a High and Low Fluoride Water Village in China. Fluoride., 44(4): 191–194.
37. Bhatnagar M., Rao P., Saxena A., Bhatnagar R., Meena P., Barbar S., Chouhan A. and Vimal S. (2006). Biochemical Changes in Brain and Other Tissues of Female Mice from Fluoride in Their Drinking Water. Fluoride, 39(4): 280–284.
38. Vani L. M. and Reddy K. P. (2000). Effects of Fluoride Accumulation on Some Enzymes of Brain and Gastrocnemius Muscle of Mice., 33: 17-26.
39. Shah S.D. and Chinoy N.J. (2004). Adverse Effects of Fluoride and/or Arsenic on the Cerebral Hemisphere of Mice and Recovery by Some Antidotes. Fluoride, 37(3): 162–171.
40. Reddy P.Y., Reddy K. P. and Kumar K. P. (2011). Neurodegenerative Changes in Different Regions of Brain, Spinal Cord and Sciatic Nerve of Rats Treated with Sodium Fluoride. J.M ed. Allie d. Sci., 1(1): 30-35.
41. Gao Q., Liu Y. and Guan Z. (2009). Decreased Learning And Memory Ability in Rats With Fluorosis: Increased Oxidative Stress and Reduced Cholinesterase Activity in The Brain. Fluoride, 42(4): 277-285.