Middle-East Journal of Scientific Research 16 (7): 1019-1026, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.16.07.12003

Does Vitamin E Protect Against Sodium Fluoride Toxicity on the Cerebellar Cortex of Albino Rats?

[1]Abdulmonem Al-Hayani, [1,3]Eman Badawi Elshal,
[1,4]Iman Hussein Abdel Aal and [2]Eida Al-Shammeri

[1]Department of Anatomy, Faculty of Medicine,
[2]Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
[3]Department of Anatomy, Faculty of Medicine, Al-Azhar University,
[4]Ain Shams University, Egypt

Abstract: The sodium fluoride was commonly added to drinking water and tooth pastes as decay preventive ingredient. It is toxic at long term exposure and leads to some adverse effects on various organs, including the brain. This study was done to investigate the changes in the cerebellar cortex of sodium fluoride intoxicated rats and the protective role of vitamin E. Forty adult male albino rats were used in this study and were divided equally into four groups: control, vitamin E, sodium fluoride treated and prophylactic. At the end of the experiment, the cerebellar cortex of all rats was processed for light microscopic examination and immunohistochemical study. In the cerebellum of sodium fluoride treated animals some of the Purkinje cells appeared shrunken with decrease in the thickness of the molecular layer. The protected group is nearly similar in appearance to the control group. Chronic exposure to sodium fluoride induces changes in cerebellar cortex. Vitamin E has protective effect on these changes. So, dietary supplements of vitamin E are recommended.

Key words: Sodium fluoride · Cerebellar cortex · Albino rat

INTRODUCTION

Fluoride was an essential trace element from the halogen group that had protective effects against bone mineral loss. Also, it can prevent caries and enamel fluorosis. Sodium fluoride was commonly added to drinking water, tooth pastes and some mouth washes as decay preventive ingredient [1-2].

The other sources of fluoride exposure include processed beverages, food additives, rodenticides and insecticides containing fluoride compounds. Previous studies documented that fluoride becomes toxic at long term exposure and leads to some adverse effects predominantly on the skeletal system and also causes damages to kidney, liver, thyroid gland, endometrium and brain [3-5]. Fluoride accumulated in dark green vegetables and concentrated in their leaves, was extracted from the soil and water [6]. Our daily foods prepared inside Teflon (fluorinated ethylene) lined cookware increase the concentration of fluoride inside them [7].

Commercially, it was used to etch glass, ceramics and computer chips, refine metals and petroleum products, make ceramic materials more permeable, inhibit fermentation in breweries and wineries, polish aluminum and as a refrigerant and rust remover. Due to the wide exposure to fluoride from different sources, it was difficult to determine its daily consumption [8].

Chronic fluoride toxicity can alter neuronal and cerebrovascular safety, abnormal behavior patterns and metabolic lesions in the brain of experimental animals [9-11].

Experimental studies exhibited that fluoride accumulation was observed in the brain of experimental animals exposed to chronic fluoride intake and this accumulation increased as drinking water fluoride content

Corresponding Author: Eman Badawi Elshal, Department of Anatomy,
Faculty of Medicine, Al-Azhar University,

increased [12]. The long term intake of high level of fluoride in human caused neurological complications such as paralysis of limbs, vertigo, spasticity in extremities and impaired mental acuity [13]. The development of children's intelligence is adversely affected according to the level of fluoride [14].

Some studies stated that chronic exposure of pregnant and lactating mothers to high level of fluoride affect some biochemical indexes of the brain and learning memory abilities of their offspring's. Generation of free radicals, lipid peroxidation and altered anti-oxidant defense system are considered to play an important role in toxic effect of fluoride [15,16]. The fluoride caused extensive oxidative stress, excessive lipid peroxidation and reduced antioxidant enzymes activities in vivo or in vitro [17].

Non enzymatic antioxidants such as vitamin E can act to overcome the oxidative stress. Studies have reported that vitamin E can reduce lipid peroxidation caused by toxic substances [18-19]. Vitamin E had many biological functions; the antioxidant function being the most important and/or best known. Other functions include enzymatic activities, gene expression and neurological functions. The most important function of vitamin E was in cell signaling in antioxidant metabolism [20].

The previous literature regarding the histological changes in fluoride-induced neurotoxicity was very little. Thus the present study was aimed to study the histological and immunohistochemical changes occurring in the cerebellar cortex of adult male albino rats following chronic exposure to sodium fluoride (NaF) and the possible protective role of vitamin E.

MATERIALS AND METHODS

Forty adult male Wister albino rats (*Rattus norvegicus*), weighing 250-350 gm, were used in this study. They were housed in King Fahd Medical Research center (KFMRC). All animals were housed for one week in suitable cages for acclimatization on the laboratory conditions before the experiment. The duration of the experiment was six weeks. Fresh water *adlibitum* and standard rodent food pellets were always available. The animals were divided equally into the following four groups:

Group I (Control Group): Each animal received 1ml normal saline once daily by oral intubation.

Group II (Vitamin E Group): Each animal received vitamin E which was obtained in the form of capsules, each capsule contained 400 mg vitamin E: EVIT®400 Soft Gelatin Capsules, Manufactured by: European Egyption Company, Packed by: Batterjee pharma, Jeddah, KSA. The dose was calculated according to a previous study [21] in a dose equivalent to the human therapeutic dose. The content of the capsule was evacuated in 5 ml corn oil and each rat was given daily 1 ml according to its weight by oral intubation.

Group III (NaF Treated Group): Each animal received 20 mg/ kg sodium fluoride orally by gastric tube once daily [22]. Sodium fluoride was obtained in the form of powder from Abu Aljaddal Company for chemical and medical trading, Jeddah, Saudi Arabia.

Group IV (Prophylactic Group): Each animal received vitamin E at the same dose and route as in group II concomitantly with the NaF at the same dose and route of group III.

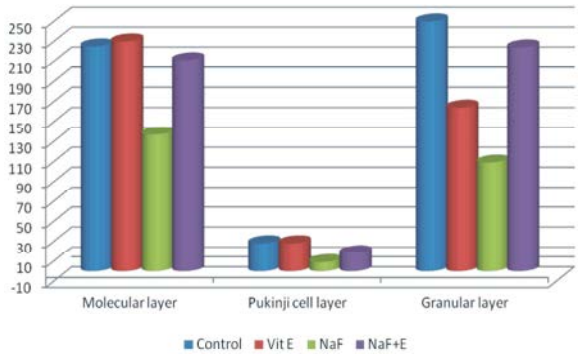
The brain was dissected and then the cerebellum was taken and prepared for light microscopic study. The specimens were fixed in 10% neutral-buffered formalin and processed for preparation of paraffin section. Sections were cut and stained with hematoxylin and eosin (H&E) for general examination and Glees and Marsland's silver stain for cytoskeleton demonstration [23].

Immunohistochemical Study

For Glial Fibrillary Acidic Protein (GFAP): After fixation in 10%, neutral formalin for 2 days, dehydration, clearing and embedding in paraffin soon followed. Paraffin section were cut at 5 µm and stained with modified avidin-biotin peroxidase technique for Glial Fibrillary Acidic Protein (GFAP) to demonstrate the astrocytes. Primary antibodies were purchased from DAKO Agilent Technologies Company Sections underwent deparaffinization and hydration. They were treated with 0.01 M citrate buffer (pH 6.0) for 10 minutes to unmask antigen. Then, they were incubated in 0.3% H_2O_2 for 30 minutes to abolish endogenous peroxidase activity before blocking with 5% horse serum for 1-2h. Slides were incubated with the primary antibody (1:100 monoclonal mouse anti GFAP) at 4co for 18-20h, then washed and incubated with biotinylated secondary antibodies and then with avidin-biotin complex. Finally, sections were developed with 0.05% diaminobenzidine slides, were counterstained with hematoxylin, dehydrated, cleared and mounted. GFAP positive cells appeared brown and nuclei appeared blue [24].

Table 1: The mean thickness of Molecular, Purkinje and Granular layers of cerebellar cortex in different groups (Control, Vitamin E, NaF and NaF + Vit. E)

	Control	Vitamin E	NaF	NaF+ E
Thickness of Molecular layer Mean ± SD	225±33	230±61	137±17	211±42
Thickness of Purkinje layer Mean ± SD	28±3	28±3	9±2	18±2
Thickness of Granular layer Mean ± SD	251±36	164±28	109±19	224±43



Histogram 1: The mean thickness of Molecular, Purkinje and Granular layers of cerebellar cortex in different groups (Control, Vitamin E, NaF and NaF + Vit. E)

Morphometric and Statistical Studies: The image analyzer computer system (Olympus) in the Anatomy Department, Faculty of Medicine, King Abdulaziz University was used to measure the mean thickness of the molecular, granular and Purkinje cell layer in each group. The recorded data for each group were analyzed using the SPSS program.

RESULTS

The Control and Vitamin E Group (Group I & II): Examination of H&E sections of the control cerebellar cortex as well as vitamin E-treated group showed the normal histological structure. They showed three apparent layers from outside inwards; the molecular layer, the Purkinje cell layer and the granular cell layer (Fig.1). The outer molecular layer was formed of nerve fibers with few scattered stellate and basket cells. The flask shaped Purkinje cells layer were arranged in one row between the granular and molecular layers. They showed large rounded nuclei with apparent nucleoli and basophilic Nissel's granules in their cytoplasm. The granular layer consisted of closely packed numerous small granular cells with deeply stained rounded nuclei (Figs.1, 2, 5).

By Glees and Marsland's silver stain, the molecular layer showed regularly arranged axons of granular cells and dendrites of Purkinje cells (Fig. 3). The cytoplasm of Purkinje cells showed homogenous brown stained cytoplasmic cytoskeletal elements (Figs. 3, 6).

Immunohistochemical staining for Glial Fibrillary Acidic Protein (GFAP) showed GFAP positive cells in the granular layer and occasionally in the molecular layer (Figs. 4, 7).

Group III (NaF Treated Group): Examination of H&E stained sections of cerebellar cortex of sodium fluoride treated group showed that most of the Purkinje cells were

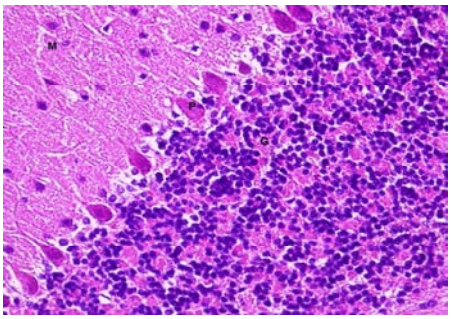


Fig. 1: A photomicrograph of cerebellar cortex of Group I showing molecular cell layer (M), Purkinje cell layer (P), and granular cell layer (G). H&E X 400

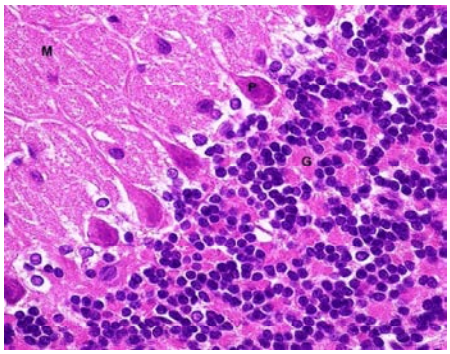


Fig. 2: A photomicrograph of cerebellar cortex of Group I showing Purkinje cell layer (P) arranged in one row between the granular and molecular layers. They have large pyriform or flask shaped cell bodies. H&E X 600

absent and some of them were present in one or 2 rows. They were shrunken with ill defined cytoplasmic processes and irregular architectures. Some of them have deep homogenous cytoplasm while others have vacuolated cytoplasm with absence of Nissel's granules.

Fig. 3: A photomicrograph of a section in the cerebellar cortex of Group I showing regular nerve fibers in the molecular layer and Purkinje cells with homogenous cytoplasmic cytoskeletal elements (→). Glees & Marsland's silver stain X400

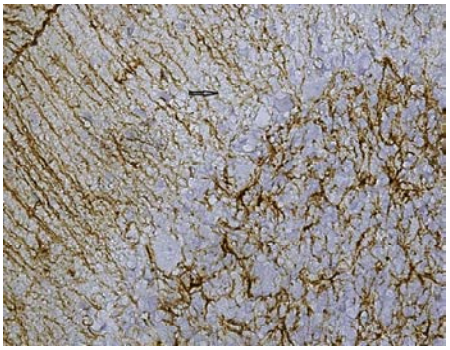


Fig. 4: Immunohistochemical staining for (GFAP) positive cells in the cerebellar cortex of Group I showing scattered positive cells in the molecular and granular layers (→). X 400

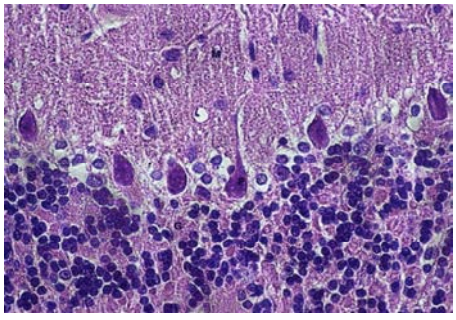


Fig. 5: A photomicrograph of a section in the cerebellar cortex of Group II showing the outer molecular layer (M) formed of nerve fibers with few scattered stellate and basket cells. The Purkinje layer (P) has large pyriform or flask shaped cells. Also note the granular layer (G). H&E X400

The molecular layer and granular were less in thickness and had multiple vacuolated areas. The cells in the granular layer were small oval or rounded with deeply stained nuclei (Fig. 8).

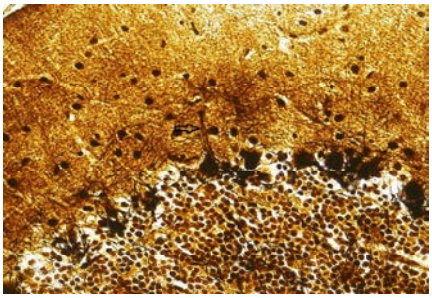


Fig. 6: A photomicrograph of a section in the cerebellar cortex of Group II showing the cytoplasm of Purkinje cells as homogenous brown stained cytoplasmic cytoskeletal elements (→). Glees & Marsland's silver stain X 400

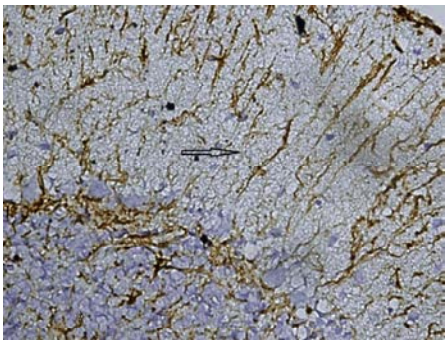


Fig. 7: Immunohistochemical staining for (GFAP) positive cells in the cerebellar cortex of Group II showing scattered positive cells in the molecular and granular layers (→). X400

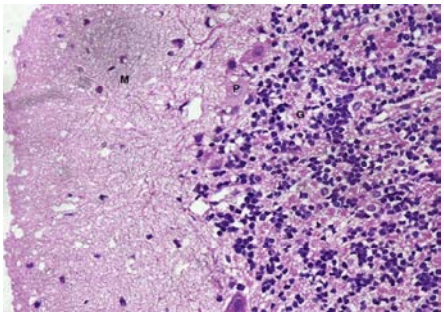


Fig. 8: A photomicrograph of a section in the cerebellar cortex of Group III showing absence of most of the Purkinje cells (P). Some of them present in one or 2 rows. They are shrunken with ill-defined cytoplasmic processes and absence of Nissel's granules. The molecular layer (M) is less in thickness. H&E X 400

Glees and Marsland's silver stained sections showed irregular arranged nerve fibers in the molecular layer, while the Purkinje cells showed

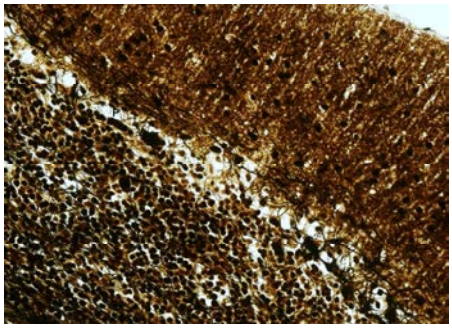


Fig. 9: A photomicrograph of a section in the cerebellar cortex of Group III showing few Purkinje cells which lost its pyramidal shape. Note the irregular arrangement of nerve fibers in the molecular layer. Glees & Marsland's silver stain X 400

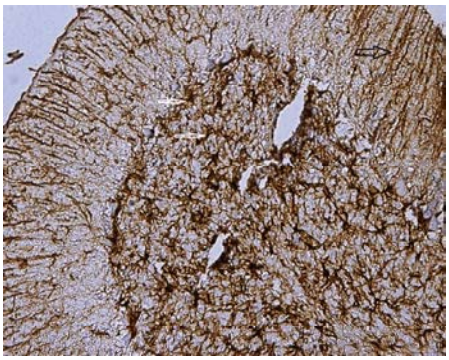


Fig. 10: Immunohistochemical staining for (GFAP) positive cells in the cerebellar cortex of Group III showing increase in the positive cells (→) mainly in the molecular layer. X 400

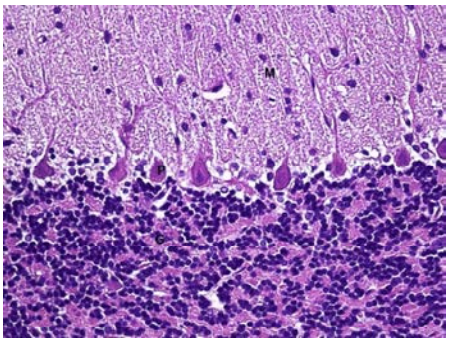


Fig. 11: A photomicrograph of a section in the cerebellar cortex of Group IV showing the Purkinje (P), molecular (M) and granular (G) layers. Few cells appear distorted with deeply stained nuclei and cytoplasm. H&E X400

increased accumulation of the cytoplasmic cytoskeletal elements that indicated deep argyrophilia (Fig. 9).

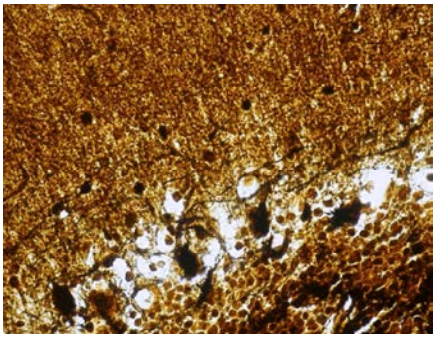


Fig. 12: A photomicrograph of a section in the cerebellar cortex of Group IV showing regularly arranged nerve fibers in the molecular layer. Glees & Marsland's silver stain X 400

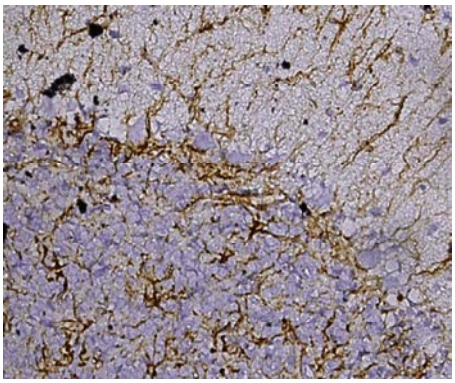


Fig. 13: Immunohistochemical staining for (GFAP) positive cells in the cerebellar cortex of Group IV showing few scattered positive cells in the molecular and granular layers. X 400

Immunohistochemical staining for Glial Fibrillary Acidic Protein (GFAP) in the cerebellar cortex of fluoride treated group showed positive immunoreaction in the cytoplasm. Also, in NaF treated group, GFAP positive cells were more numerous and appeared larger in the three cortical layers (Fig. 10).

Group IV (Prophylactic Group) Sodium Fluoride and Vitamin E treated Group: Examination of H&E stained sections of the cerebellar cortex of prophylactic group showed monolayer arrangement of Purkinje cells with mild disorganization. Few Purkinje cells were still affected in between many apparently normal cells with central vesicular nuclei. Some of them were surrounded with vacuolated cytoplasm. The molecular and granular layers were more or less similar to the control (Fig. 11).

Glees and Marsland's silver stained sections of the cerebellar cortex appeared nearly more or less as the

control (Fig. 12). Immunohistochemical staining for Glial Fibrillary Acidic Protein (GFAP) showed positive immunostaining in the cytoplasm (Fig. 13).

Morphometric and Statistical Studies: A significant decrease in the mean thickness of the cerebellar cortex cell layers in fluoride treated group was detected as compared to the control group (Table 1 and Histogram 1).

DISCUSSION

Fluoride is widely distributed in nature in many forms and its compounds are used extensively. Fluoride in small doses had prophylactic influence by inhibiting dental caries while in higher doses it causes dental and skeletal fluorosis [25]. Fluoride is absorbed completely and quickly from the gastrointestinal tract and affects important organs [26].

The cerebellum is responsible for smooth and accurate movements and any lesion in it is manifested as signs of cerebellar ataxia [27].

In this work, NaF induced damage and disorganization in Purkinje cells. Purkinje cells were arranged in one or two layers as compared to the control group. They were shrunken with ill defined cytoplasmic processes and some of them had vacuolated cytoplasm with absence of Nissel's granules. The molecular and granular layers were less in thickness and had multiple vacuolated areas. The cells in the granular layer were small, oval or rounded with deeply stained nuclei. This was in agreement with the study which confirmed that the ingested fluoride accumulated in the cerebellum, induced neurotoxicity, cell damage and even cell death [28]. Similar findings were observed by researchers, who found that chronic fluoride intoxication caused neuro-degenerative changes in rats especially in the early stages of life [29,30]. There were histopathological changes in the brain in the form of chromatolysis of Nissele's granules and gliosis of young fluoride-intoxicated rats. The neuronal loss in fluoride intoxicated rabbits was accompanied by increased glial cells' number as a compensatory response to neuronal damage by fluoride [31, 32]. The previous results seemed to be due to damage to the system associated with structure and functional biosynthesis of cell proteins [33].

Glial Fibrillary Acidic Protein (GFAP), is an intermediate filament protein expressed by numerous cell types especially in the astrocytes [34]. It was known as a specific marker of mature astrocytes of the central nervous system. The astrocytes have an important role in the repair and scarring process of the brain following traumatic injuries. Its expression is essential for normal white matter morphology and blood brain barrier [35, 36]. Central nervous system damage due to any neurotoxic substances causes rapid reaction of the astrocytes which is expressed by GFAP protein marker for astrogliosis [35]. In this study the increased positive expression of GFAP was due to neuronal damage caused by fluoride administration. This finding was supported by other investigators who explained that hypertrophy and increased content of gliofilaments and organelles occur as a compensatory mechanism after neurodegeneration or as a matter of cell death or a neuroprotective response [37].

Vitamin E was the most important lipophilic antioxidant and resides mainly in the mitochondria thus helping to maintain membrane stability. Also, it decreased the cell death which is due to the oxidants and free radicals in different cells [38]. The ingestion of vitamin E protects against lipid peroxidation most efficiently through its anti-oxidant action. The oxidative stress plays an important role in mediating apoptosis through mitochondrial dysfunction [19]. In this study, a concomitant administration of vitamin E and sodium fluoride in the protected group significantly reduced the damage induced by sodium fluoride in cerebellar cortex of rats, but not completely recovering the histopathological changes to normal. Also, diminished GFAP immunoreaction was observed when compared with those of fluoride treated group. Vitamin E has protective effect on these changes. So, dietary supplements of vitamin E are recommended.

REFERENCES

1. Pendrys, D.G., 2001. Fluoride ingestion and oral health. Nutrition Nov-Dec., 17(ll-12): 979-980.
2. Abed, K.F. and S.S. Alwakeel, 2007. Mineral and Microbial Contents of Bottled and Tap Water in Riyadh, Saudi Arabia: Middle-East Journal of Scientific Research, 2(3-4): 151-156.
3. Wang, J., Y. Ge, H. Ning and S. Wang, 2004. Effects of high fluoride and low iodine on biochemical indexes of the brain and learning-memory of offspring rats. Fluoride. 37(3): 201-208.
4. Xu, H., X.Q. Jin, L. Jing and G.S. Li, 2006. Effect of sodium fluoride on the expression of bcl-2 family and osteopontin in rat renal tubular cells. Biol. Trace Elem. Res., 109(l): 55-60.
5. Guney, M., B. Oral, G. Take, S.G. Giray and T. Mungan, 2007. Effect of fluoride intoxication on endometrial apoptosis and lipid peroxidation in rats: Role of vitamins E and C. Toxicol., 231(2-3): 215-223.

6. Fung, K.F., Z.Q. Zhang, J.W.C. Wong and M.H. Wong, 1999. Fluoride contents in tea and soil from tea plantations and the release of fluoride into tea liquor during infusion. Environ. Pollut., 104(2): 197-205.
7. Horowitz, H.S., 1999. Proper use of fluoride products in fluoridated communities. Lancet May 1; 353(9163): 1462.
8. Lund, K., M. Refsnes, T. Sandstrom, P. Sostrand, P. Schwarze, J. Boe and J. Kongerud, 1999. Increased CD3 positive cells in bronchoalveolar lavage fluid after hydrogen fluoride inhalation. Scand. J. Work Environ. Health, 25(4): 326-334.
9. Shashi, A., 1992. Studies on alterations in brain lipid metabolism following experimental fluorosis. Fluoride Q. Rep., 25(2): 77-84.
10. Shashi, A., J.P. Singh and S.P. Thapar, 1994. Effect of long-term administration of fluoride on levels of protein, free amino acids and RNA in rabbit brain. Fluoride Q. Rep., 27(3): 155-159.
11. Varner, J.A., K.F. Jensen, W. Horvath and R.L. Isaacson, 1998. Chronic administration of aluminum-fluoride or sodium-fluoride to rats in drinking water: Alterations in neuronal and cerebrovascular integrity. Brain Res., 16; 784(1 -2):284-298.
12. Mullenix, P.J., P.K. Denbesten, A. Schunior and W.J. Kernan 1995. Neurotoxicity of sodium fluoride in rats. Neurotoxicol. Teratol., 17(2): 169-177.
13. Lu, Y., Z.R. Sun, L.N. Wu, X. Wang, W. Lu and SS. Liu, 2000. Effect of high-fluoride water on intelligence in children. Fluoride, 33(2): 74-78.
14. Xiang, Q., Y. Liang, L. Chen, C. Wang, B. Chen and M. Zhou, 2003. Effect of fluoride in drinking water on children's intelligence. Fluoride, 36(2): 84-94.
15. Sharma, A. and N.J. Chinoy, 1998. Role of free radicals in fluoride-induced toxicity in liver and kidney of mice and its reversal. Fluoride, 31: S26.
16. Rzeuski, R., D. Chlubek and Z. Machoy, 1998. Interactions between fluoride and biological free radical reactions. Fluoride, 3l(l): 43-45.
17. Shan, K.R., X.L. Qi, Y.G. Long, A. Nordberg and Z.Z. Guan, 2004. Decreased nicotinic receptors in PC12 cells and rat brains influenced by fluoride toxicity~a mechanism relating to a damage at the level in post-transcription of the receptor genes. Toxicology, 5; 200(2-3): 169-177.
18. Altuntas, I., N. Delibas and R. Sutcu, 2002. The effects of organophosphate insecticide methidathion on lipid peroxidation and anti-oxidant enzymes in rat erythrocytes: Role of vitamins E and C. Hum.Exp.Toxicol., 21(12): 681-685.
19. Serbecic, N. and S.C. Beutelspacher, 2005. Anti-oxidative vitamins prevent lipid-peroxidation and apoptosis in corneal endothelial cells. Cell Tissue Res., 320(3): 465-475.
20. Bell, E.F., 1987. "History of vitamin E in infant nutrition". American Journal of Clinical Nutrition, 46(1 Suppl): 183-186.
21. Paget, G.E. and J.M. Barnes, 1964. Evalution of Drug Activites and pharmacokinetics. Acadenic Press, Vol. I: 135-136.
22. Blaszczyk, I., E. Grucka Mamczar, S. Kasperczyk and E. Birkner, 2008. Influence of fluoride on rat kidney antioxidant system: Effects of methionine and vitamin E. Biol. Trace Elem. Res., 121(1): 51-59.
23. Drury, R.A. and E.A. Wallington, 1980. Carleton's histological technique.5th ed.Oxford University Press: London.
24. Cattoretti, G., S. Pileri, C. Parravicini, M.H. Becker, S. Poggi, C. Bifulco, G. Key, L.D'Amato, E. Sabattini, E. Feudale 1993. Antigen unmasking on formalin-fixed, paraffinembedded tissue sections. J. Pathol., 171(2): 83-98.
25. Shanthakumari, D., S. Srinivasalu and S. Subramanian, 2004. Effect of fluoride intoxication on lipidperoxidation and antioxidant status in experimental rats. Toxicol., 204(2-3): 219-228.
26. Amira, S., S. Soufane and K. Gharzouli, 2005. Effect of sodium fluoride on gastric emptying and intestinal transit in mice: Exp. Toxicol. Pathol., 57(1): 59-64.
27. Fitz Gerald, M.J.T. and J. Folan Curran, 2002. linical neuroanatomy and related neuroscience. 5th ed. W.B. Saunders.
28. Trabelsi, M., F. Guermazi and N. Zeghalc, 2001. Effect of fluoride on thyroid function. Fluoride, 34(3): 165-173.
29. Hirai, T., M. Mizutani, K. Ochiai, T. Umemura and C. Itakura, 1999. Distal axonopathy does not occur without neurofilament accumulation in gamma diketone neuropathy: Comparative studies of normal and neurofilament-deficient quail. Acta Neuropathol. 97(6): 552-556.
30. Shivarajashankara, Y.M., A.R. Shivashankara, P. Gopalakrishna Bhat and S. Hanumanth Rao, 2001. Effect of fluoride intoxication on lipid peroxidation and antioxidant systems in rats. Fluoride, 34(2): 108-113.
31. Shashi, A., 2003. Histopathological investigation of fluoride-induced neurotoxicity in rabbits. Fluoride, 36(2): 95-105.

32. Ge, Y., H. Ning, S. Wang and J. Wang, 2005. Effects of high fluoride and low iodine on brain histopathology in offspring rats. Fluoride, 38(2): 127-132.
33. Ratan, R.R., T.H. Murphy and J.M. Baraban, 1994. Oxidative stress induces apoptosis in embryonic cortical neurons. J. Neurochem. 62(1): 376-379.
34. Der Perng, M., M. Su, S.F. Wen, T. Gibbon, A.R. Prescott, M. Brenner and R.A. Quinlan, 2006. The Alexander disease-causing glial fibrillary acidic protein mutant, R416W, accumulates into Rosenthal fibers by a pathway that involves filament aggregation and the association of alpha B-crystallin and HSP27. Am. J. Hum. Genet. 79(2): 197-213.
35. Eng, L.F. and R.S. Ghirnikar, 1994. GFAP and astrogliosis. Brain Pathol., 4(3): 229-237.
36. Li, D.R., T. Ishikawa, D. Zhao, T. Michiue, L. Quan, B.L. Zhu and H. Maeda 2009. Histopathological changes of the hippocampus neurons in brain injury. Histol. Histopathol., 24(9): 1113-1120.
37. Borlongan, C.V., M. Yamamoto, N. Takei, M. Kumazaki, C. Ungsuparkorn, H. Hida, P.R. Sanberg and H. Nishino, 2000. Glial cell survival is enhanced during melatonininduced neuroprotection against cerebral ischemia. FASEB J., 14(10): 1307-1317.
38. Sramanathan, K., M. Anusuyadevi, S. Shila and C. Panneerselvam, 2005. Ascorbic acid and alpha-tocopherol as potent modulators of apoptosis on arsenic induced toxicity in rats. Toxicol. Lett., 10; 156(2): 297-306.