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Research Article

**IMPACT OF SODIUM FLUORIDE ON SPINAL CORD'S  
GREY MATTER ON ADULT MALE ALBINO RATS**<sup>1</sup>Madiha Amjad, <sup>2</sup>Ayesha Khalid, <sup>1</sup>Memoona Ansari<sup>1</sup>Avicenna Medical College Lahore<sup>2</sup>King Edward Medical College Lahore**Article Received:** December 2019 **Accepted:** January 2020 **Published:** February 2020**Abstract:**

**Objectives:** Fluoride is among the elements which is reactive as well as toxic. The exposure of humans to this element is very common from different sources like mouth rinses, normal water for drinking, supplements of diet and toothpastes. The aim of this study is to investigate the impact of NaF alone or in combination with green tea on spinal cords grey matter of albino rats.

**Methodology:** We examined the sections of spinal cord with the utilization of light as well as electron microscopy. Immuno-histochemical evaluations for the manifestation of Caspase-3 and GFAP (Glial Fibrillary Acidic Protein). We used the AGE (Agarose Gel Electrophoresis) for the fragmentation of DNA.

**Results:** The results of this research work showed that sodium fluoride is the cause of production of neurodegeneration, hemorrhage, vacuolation with abnormal vessels of blood and important rise in the size and amount of the astrocytes. Additionally, neurons displayed an important reduction of their content of Nissl's granules and significant enhanced Caspase-3 expression.

**Conclusion:** Green tea employed a defensive impact in opposition neurotoxicity induced by sodium fluoride. The findings of this research work add to acknowledged toxic impacts of sodium fluoride.

**KEYWORDS:** Agarose Gel Electrophoresis, Green Tea, Hemorrhage, Astrocytes, Fragmentation, Spinal Cord, Fluoride.

**Corresponding author:****Madiha Amjad,**

Avicenna Medical College Lahore

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**INTRODUCTION:**

One of the important trait of fluoride is that it is reactive as well as toxic. There are different sources through which humans are exposed to this fluoride. There is an important proportion of fluoride is available in our routine foodstuff. The most important source of this element is the sources of normal drinking water which is responsible for the greater than sixty percent of the total intake of fluoride. Fluoride is a toxic element not only for the skeletal tissues but also for the tissues which are non-skeletal like liver, kidneys and brain. Fluoride has the ability to cross the barrier of brain's blood and is responsible for the adverse impacts on the metabolism of brain cells, enzymes, oxidant status, neuro-transmitters and it can also cause bad impacts on the functions of mental ability. This is also responsible for different morphological alterations in different sub-regions of brain.

Spittle observed that among various signs of chronic toxicity of fluoride are the muscle spasms, not voluntary twitching sensations in fingers and feet which may show an impact on spinal cord. Different research works on the neuro-protective impacts of this green tea showed that this act as anti-oxidants as well as modulators of intra-cellular neuronal metabolism and signaling, survival of cells and functions of mitochondria. Therefore, there is much consideration on these impacts. This very research work carried out to examine the impact of sodium fluoride on the grey matter of spinal cord to check the protective role of green tea.

**METHODOLOGY:**

We obtained 24 male albino rats having mean weight of 200 grams for this research works. We kept them with extreme care in cages. We take care the laboratory standards for those rats. We divided the animals into 3 groups. Group-1 was the group of controls with 12 rats. We subdivided this again into 2 subgroups; Subgroup-1a which was negative control sub-group consisted total 6 rats that obtained distilled of 2.40 ml/rat/day for complete 28 eight days with gastric intubation. Sub-group 1b was the positive control green tea sub-group contained 6 rats that obtained the extract of green of 10.0 mg/kg/day for complete twenty-eight days with gastric intubation. There were 6 rats in Group-2 that obtained NaF at dose of 12.0 mg/kg/day (known as NaF treated group) for complete twenty-eight days with the gastric intubation. Group-3 (NaF plus green tea group) contained 6 rats that obtained extract of green tea thirty minutes' prior administration of sodium fluoride at same doses and the duration of the other described groups.

We obtained the NaF from a company in shape of white powder. We prepared the solution of sodium fluoride with distilled water with a specific quantity of both. We also obtained the tablets of green tea which was of 200 mg. We measured the weight of all rats at the initial and final stage of experiment. We exposed the spinal cord after anesthesia with standard procedure and we removed the cord from the spinal canal. There was a clear observance of the cord's cervical and lumbar enlargements in the lumber and cervical regions as presented in Figure-1.

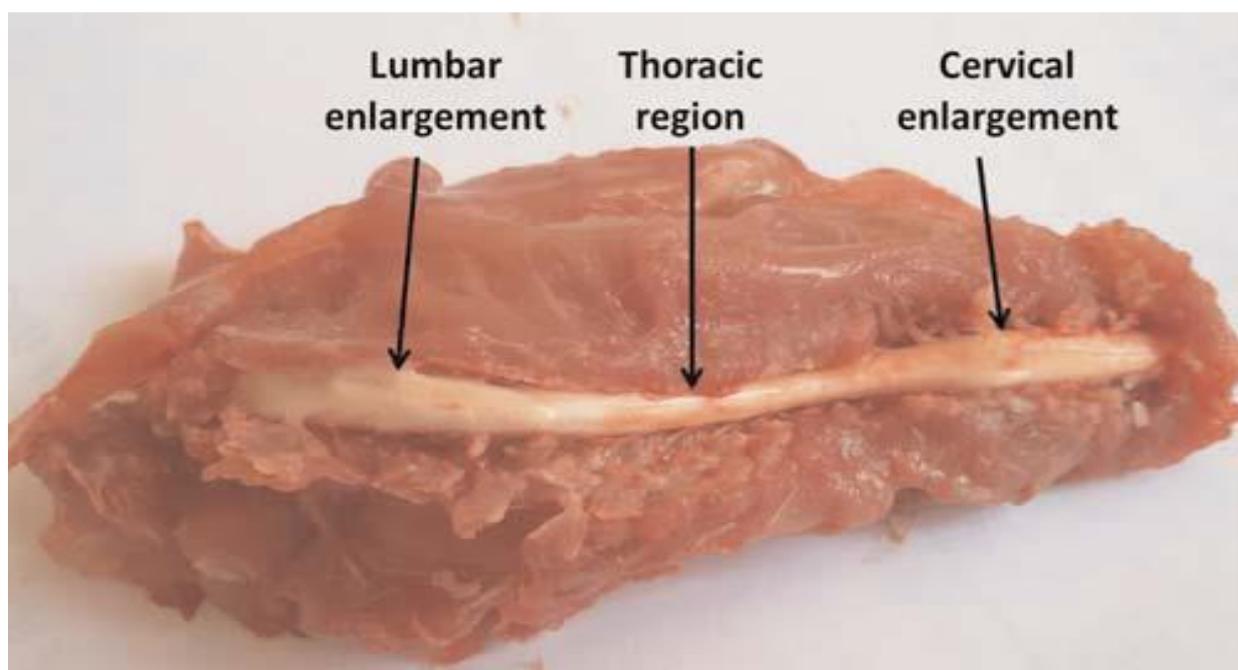


Figure 1

We extracted the cervical and thoracic portions of spinal cord and then preserved in 10.0% neutral buffered formaldehyde for complete one day. We cut the sections of 3 to 5  $\mu\text{m}$  thickness by microtome and stained by H and E (Hematoxylin and Eosin stain) for examination through histology, used silver stain for the argyrophilic cells and TB stain (Toluidine Blue) for the Nissl's granules. Immunohistochemical staining for the Caspase-3 as an identifier for apoptosis conveyed in nucleus and cytoplasm was performed with the utilization of mouse monoclonal Caspase-3-antibody, GFAP as an identifier for gliosis expressed in only cytoplasm of the astrocytes utilizing the rabbit polyclonal GFAP-antibody.

We used the small dissected immersed sections of spinal cords with the help of electron microscopy which were TB stained. We analyzed the 5 various stained sections of spinal cord's ventral horn from 5 rats with the utilization of the Image J Version 1.470 software for the measurement of astrocytes number in the H and E stained sections, intensity of color of Nissl's granules in TB stained sections and

astrocytes percent area in the GFAP immune-stained sections. We used the AGE (Agarose Gel Electrophoresis) for the detection of the fragmentation of the DNA. We conducted this research work in the Animal Department of our institute. P value of less than 0.050 was the significant. We used the SPSS V.20 for the statistical analysis of the collected information. We performed the non-parametric tests due to the very small size of samples.

### RESULTS:

The samples of negative control sub-group "Subgroup 1a" displayed an important rise in the weight of body at experiment end in comparison to their weight at the initial stage of the experiment. Animals of the positive control sub-group "Subgroup 1b", treated group "Group-2" and protected group "Group-3" displayed an important reduction in the weight of body at experiment end. But the percentage of the reduction in the weight of body of protected group was much high as compared to the reduction in the weight of body of both Subgroup 1b and Group-2 as shown in Figure-2.

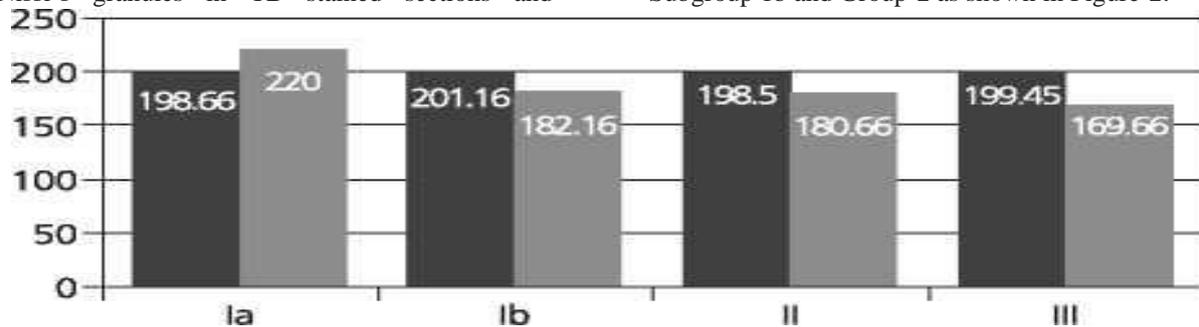
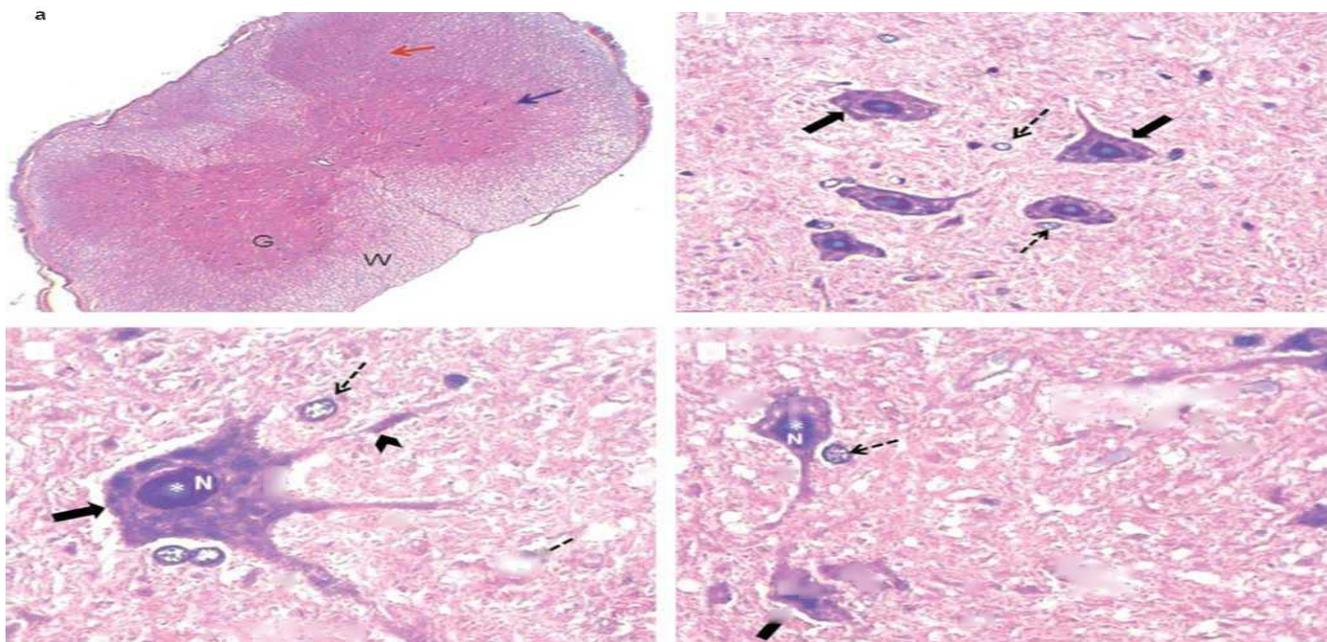


Figure 2 (a, b, c & d)



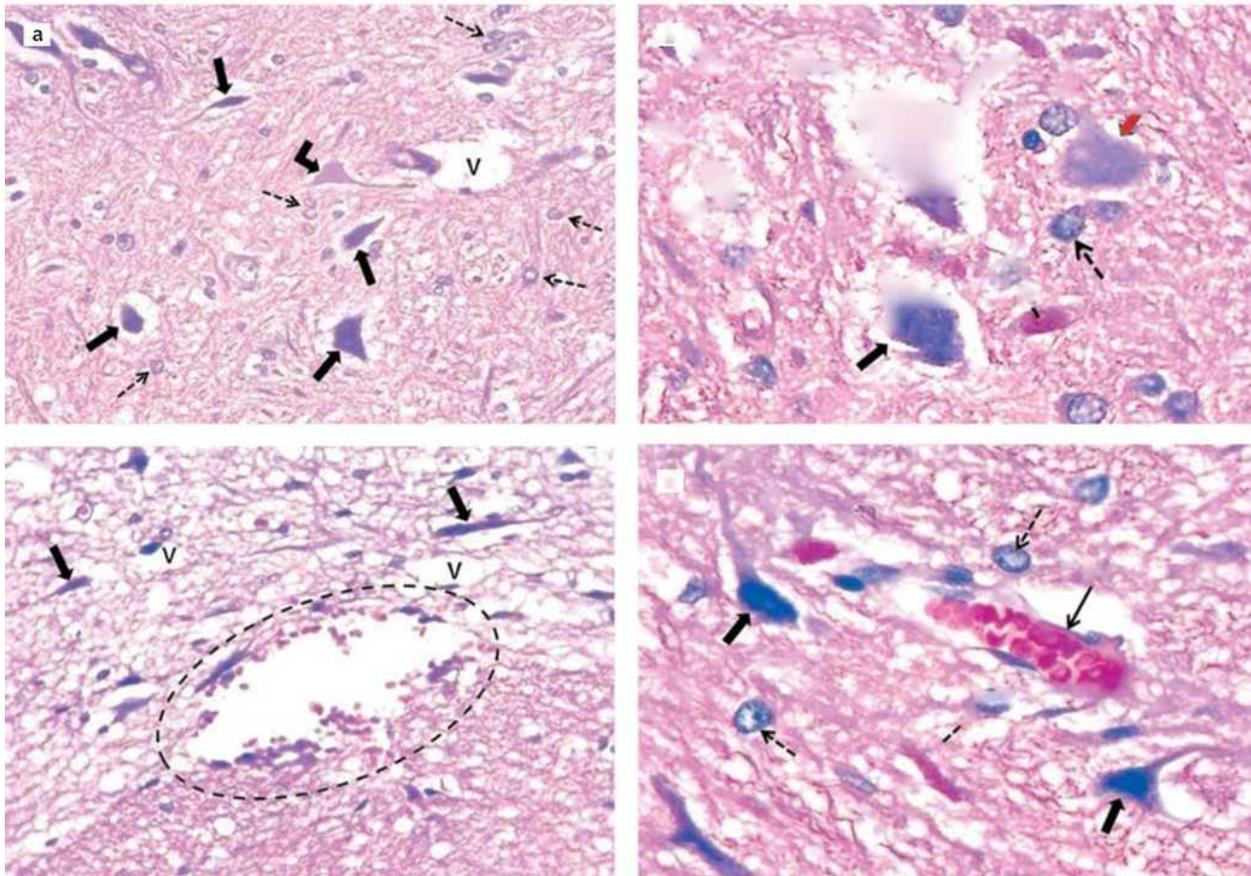


Figure 3 (a, b, c & d)

In the findings of light microscopy in H and E stained sections, transverse section of cervical region of spinal cord of group of controls was viewed as a light white matter at outer side and dark grey matter at inner side. We divided the H shaped grey matter into ventral and dorsal horns (Figure-3a). Every neuron in both ventral and dorsal horns was present with central nucleus with a noticeable nucleolus. Some of the cells were present with the long processes (Figure- 3b-d). We found some small thin vessels of walled blood inside the neuropil (Figure-3d) and some astrocytes present with small vesicular nuclei as presented in Figure 3b-d.

The appearance of some cells was ghost like (Figure- 4a-d). Neuropil displayed an important rise in the proportion of astrocytes ( $P < 0.010$ ) as compared to the group of controls (Figure- 4a-d and Figure-5).

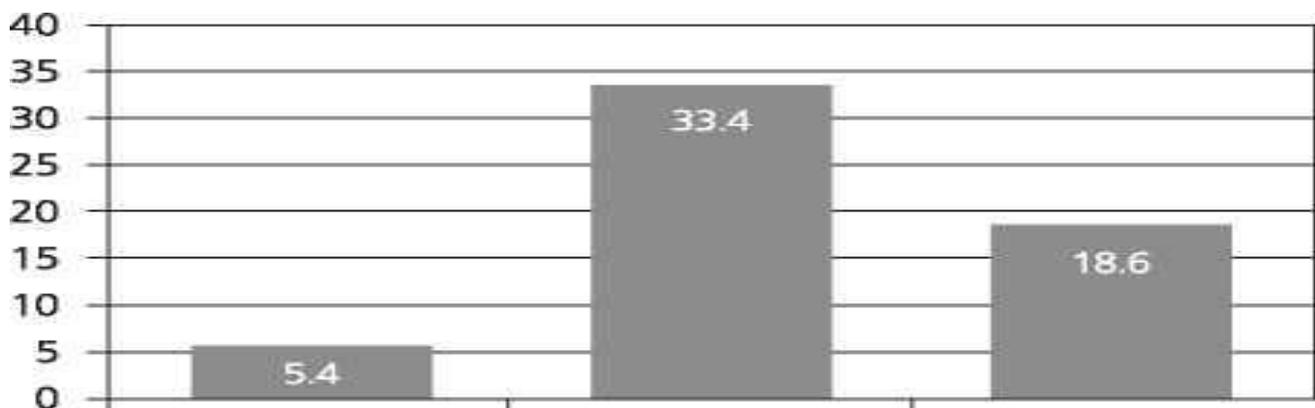


Figure 4: Number of astrocytes in cervical sections of the rat spinal cord.

Some neurons displayed loss of nuclear elaboration whereas remaining were shrunken with the cytoplasm

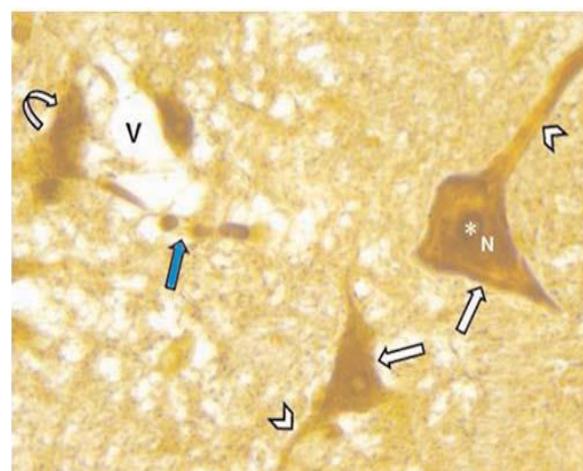
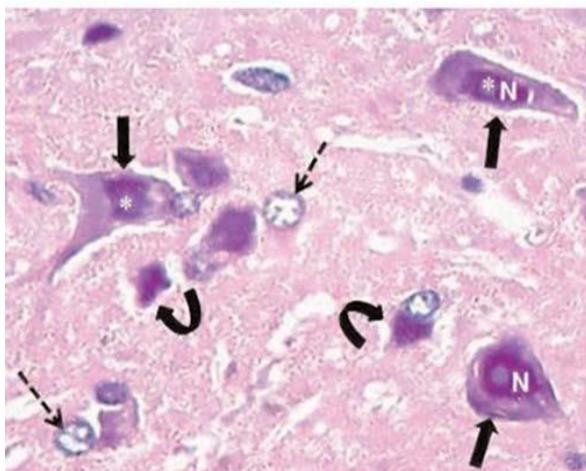
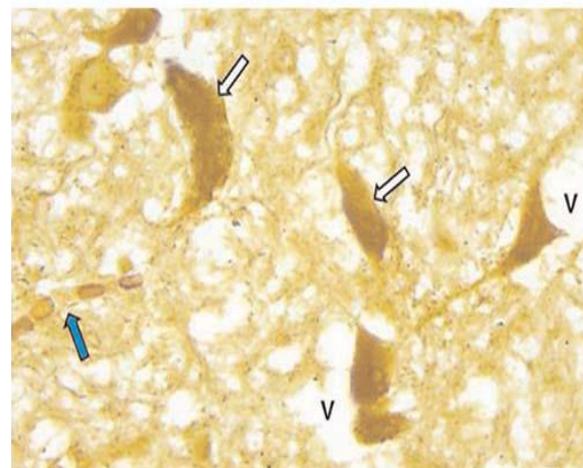
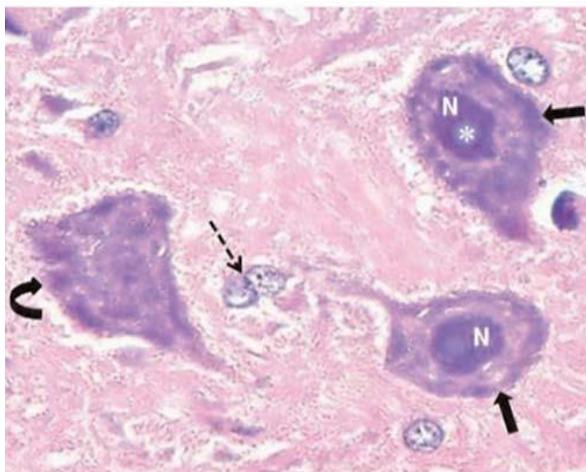
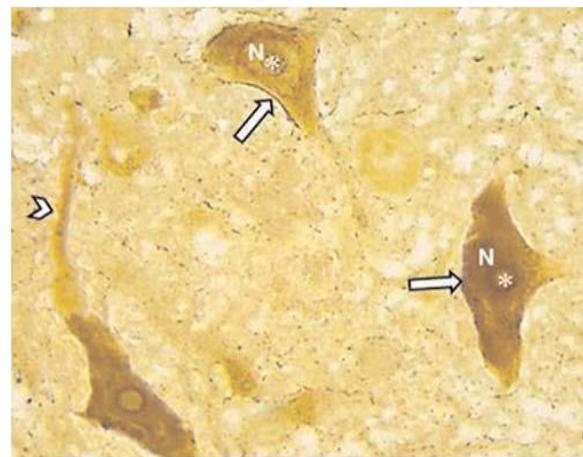
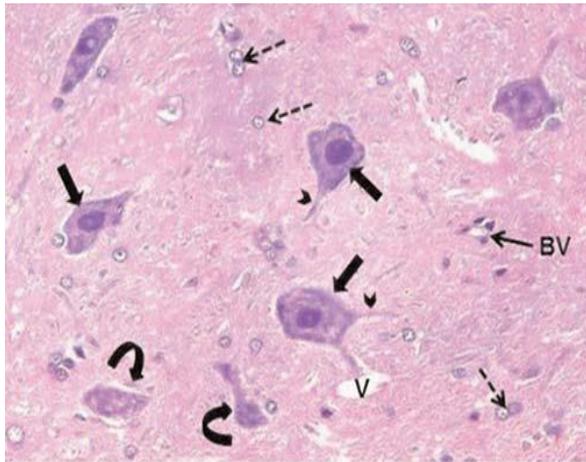


Figure 6

Figure 7

of dark color as well as loss of the details about nuclear (Figure- 6a-c). There was an important reduction in the amount of astrocytes in this group as compared to the NaF-treated group ( $P < 0.010$ ) (Figure-5 and 6a). We also found an important decrease in the amount of astrocytes in this group as compared to the group of controls ( $P < 0.010$ ) (Figure-5).

In sections which were silver-stained, the group of controls displayed an amount of large argyrophilic multipolar neurons present with cen-had long processes (Figure-7a). We also observed few degenerated axons present with segmental myelination disruption in neuropil (Figure-7b). In protected group, shrunken cells were partially degenerated and surrounding of these cells was with regions of vacuolation (Figure-7c). In sections which were TB-stained, controls group's cytoplasm of motor neurons was present with content of dense Nissl's granules which was in dark blue color (Figure-8a).

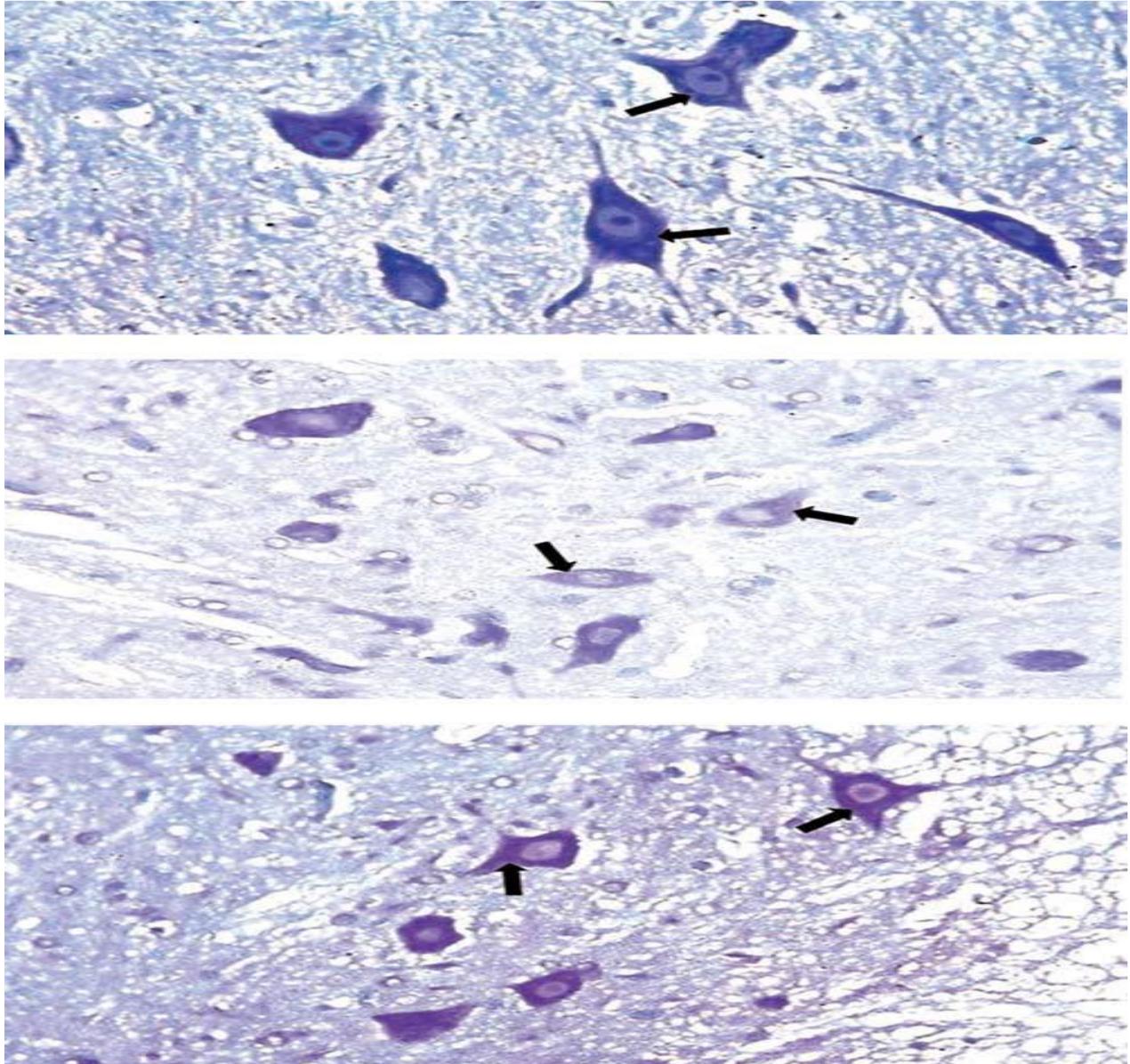


Figure 8

In group treated with NaF, cytoplasm of motor neurons displayed an important reduction of content of Nissl's granules ( $P < 0.010$ ) that was of faint blue color (Figure 8b). In the sections which were Caspase-3-staining, neurons of animals in control group displayed negative immune reaction to Caspase-3 (Figure-9a). Neurons of the animals of groups treated with NaF and protected group are present in Figure-9b and Figure-9c respectively. In the section having GFAP-staining, animals of control group displayed small astrocytes as well as faint brown color (Figure-10a).

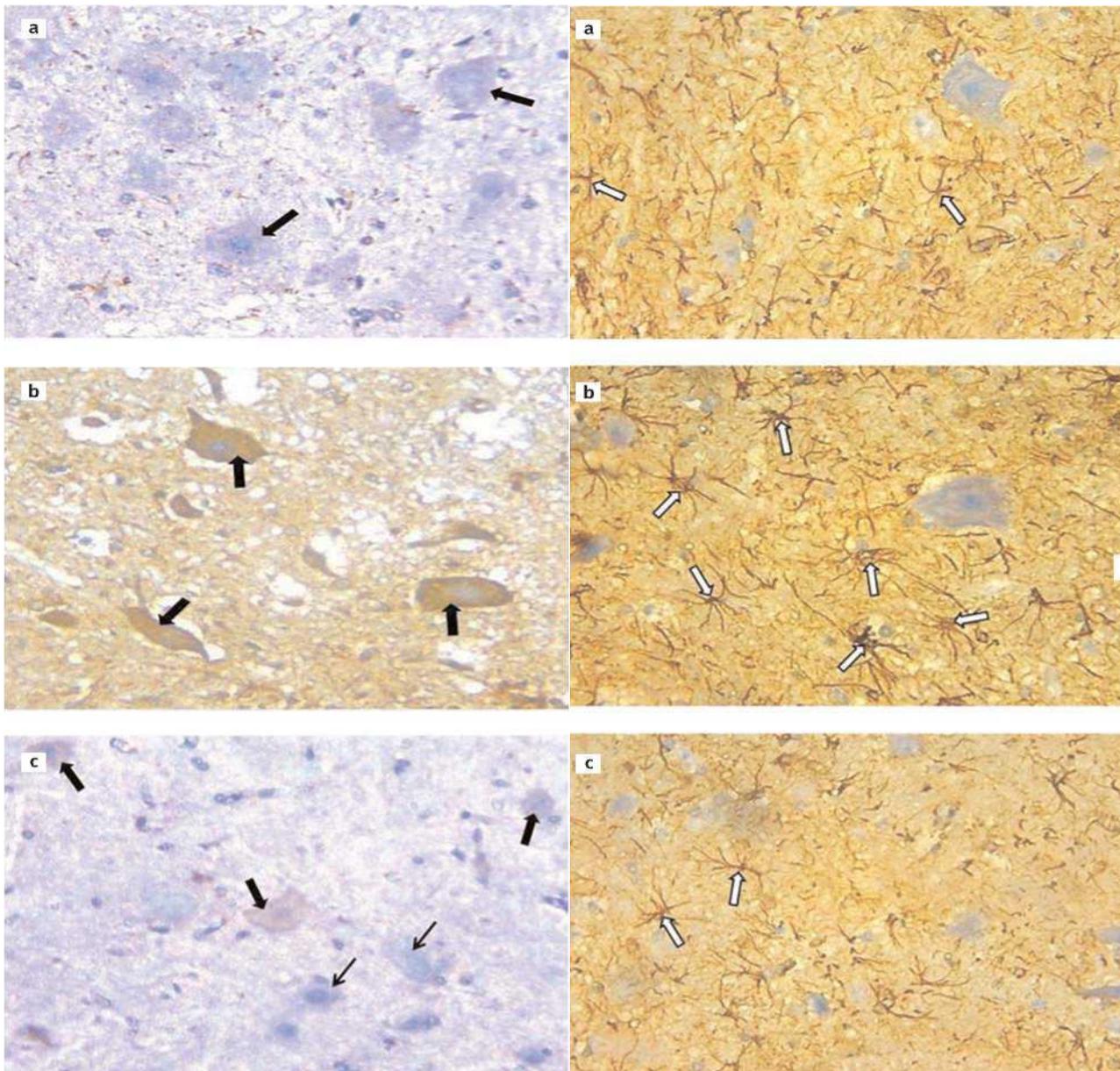


Figure 9

Figure 10

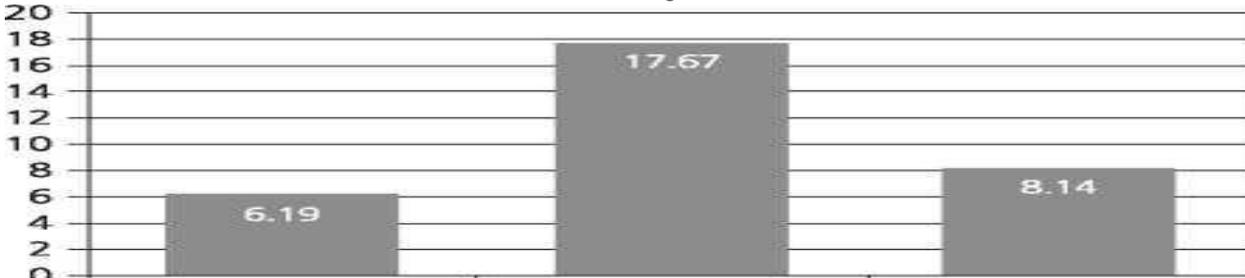


Figure 11: Mean percent area of astrocytes in cervical sections of the spinal cord.

In the group treated with NaF, neuropil displayed an important rise in astrocytes size present with the longer processes in comparison to the control group (Figure-11b and 12). In protected group, we found an important decrease in amount and size of the astrocytes ( $P < 0.0010$ ) as compared to the animals treated with the NaF (Figure-10c and 11).

In group of controls, there were many free ribosomes scattered in the cytoplasm between the organelles (Figure-12a). There could be detection of few regions of vacuolation in the cytoplasm (Figure-12a). Except for chromatin's condensation, there was normal appearance of the nuclei (Figure-12d).

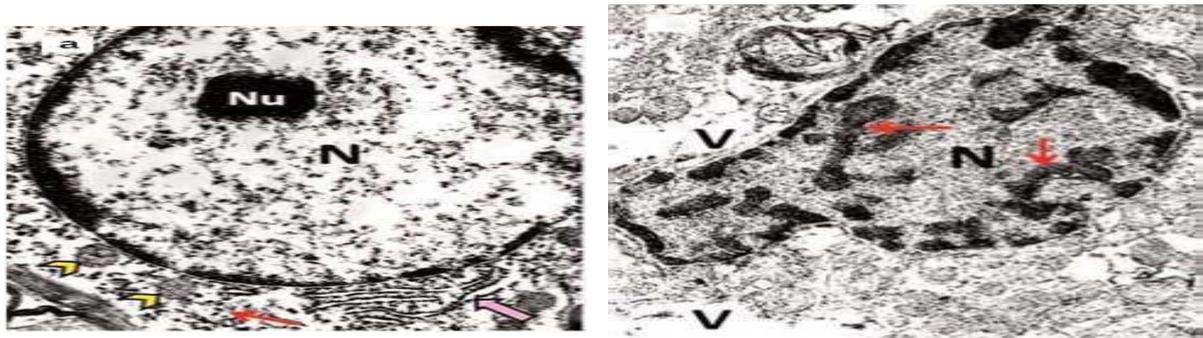


Figure 12

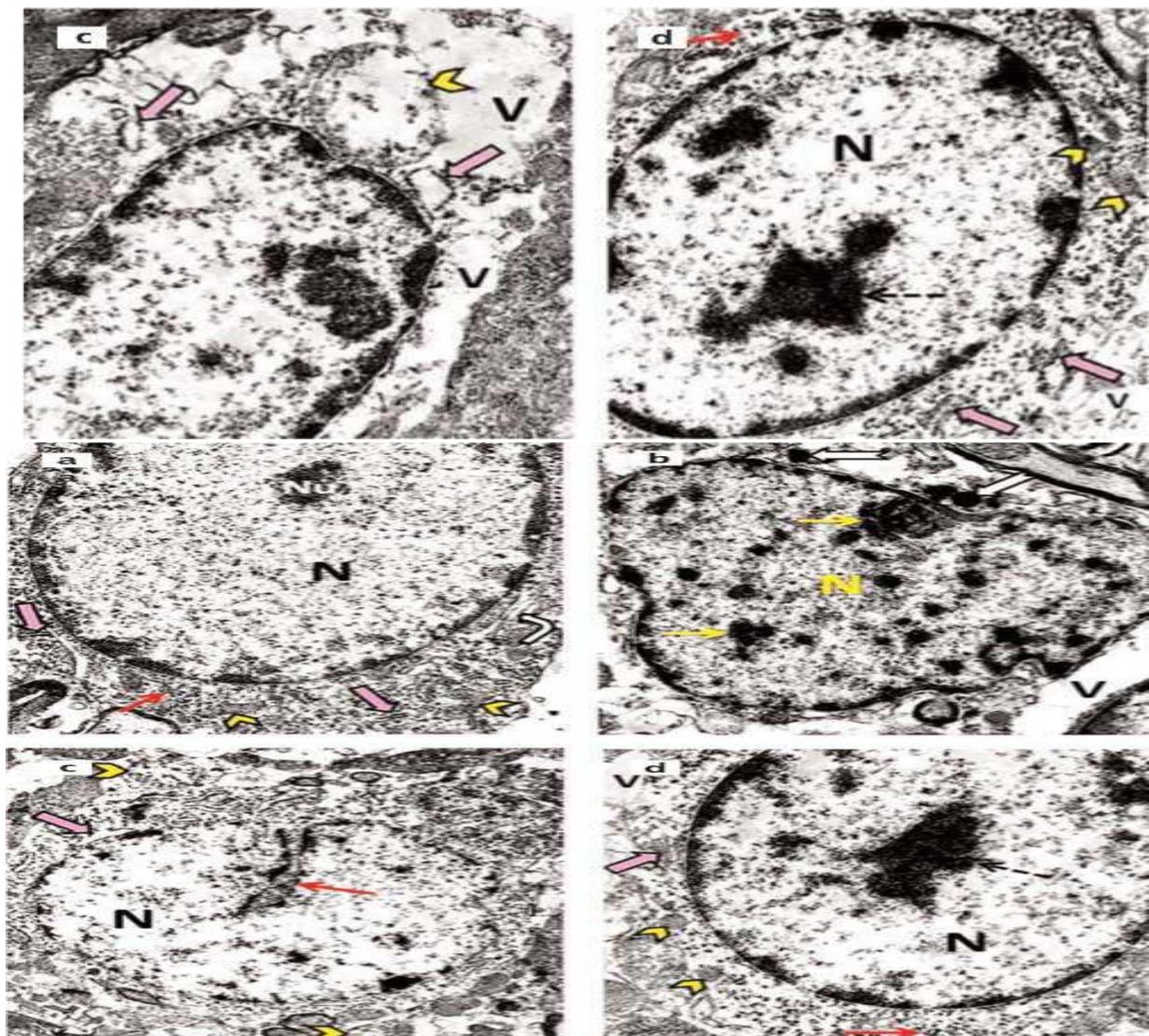


Figure 13

In the group of controls, many black granules which were free ribosomes present in scattered condition in cytoplasm between various organelles of the cell (Figure-13 a). In the group treated with NaF, cytoplasm consisted few dilated PER and swollen vacuolated mitochondria. There could be detection of few regions of vacuolation inside cytoplasm (Figure 13 b and c). There could be detection of the small regions of vacuolation in cytoplasm. Except for few chromatin's condensation, there was normal appearance of nuclei (Figure-13d). In protected group, there could be detection of apoptotic bands but it was less than the group treated with NaF.

### DISCUSSION:

Fluoride can have bad impacts on various organs of human body due to its quick absorbed nature. Researchers in whole the world thought it necessary that there is a requirement to study the impacts of fluoride on tissues of human body including CNS (Central Nervous System). In this current research work, the samples of negative control sub-group displayed an important rise in the weight of body at experiment end which is similar to the findings of the research work of Soliman. The specimens of the group treated with fluoride in this research work displayed an important rise in the weight of body at experiment end which is similar to the findings of Reddy. Trivedi stated that reduction in weight is due to low metabolism of energy and reduced synthesis of protein. Nour-Edien observed that the decrease in the weight of body is due to the direct corrosive impact of fluoride on gut and gastric mucosa. Additionally, Warren and Mitchell stated the reduction of weight due to the inhibitory impact of fluoride on metabolism of protein and activity of enzymes. This research work proved that addition of the green tea in the sodium fluoride is the case of the important reduction in the weight of body. This result is also similar with the outcome of Lu. Farooqui also explained the role of green tea in the loss of bodyweight. Cooper stated that anti-obesity impacts of green tea to the rise in thermogenesis. This current research work defined the shape and condition of the cytoplasm and nucleus of cells which is much similar to the research work of Nour-Edien and Saad El-Dien. Giri in his research work observed the hemorrhages in important organs like kidney, heart and liver of rats treated with the NaF. Milton Prabu attributed the neuro-generative impacts of NaF. NaF is also cause of the activity of synthesis of nitric oxide. This enzyme performs very important role in all the diseases of neuro-degeneration. Refsnes also described the adverse impacts of fluoride on the inhibition of various enzyme's function. Hemorrhage as viewed in neuropil in current research work was also described by the Levine. A probable elaboration for the reduced Nissl's granules in the rats treated with NaF is the accretion of free radicals that can cause the decrease in neuron's protein forming machinery also mentioned by Chen in his research work. Song stated the positive impression of Caspase-3 and Caspase-9 proteins in the liver of rats treated with NaF. Agalakova and Gusev divided the caspases into effector and initiator. Al-Hayani in his research work explained that GFAP positive cells were with

high abundance and appeared larger in rats treated with fluoride. Akinrinade stated the administration of fluoride for thirty days resulted a rise in GFAP immune-reaction. This explanation is also present in the research work of Wang. Hamdy explained in his research work that green tea is the cause of normalization of the level of elevated nitric oxide. Thangapandiyan also observed the protective impacts of green tea which may be due to the anti-genotoxicity of EGCG.

### CONCLUSION:

The results of this research work displayed the protective impact of green tea against the neurotoxicity induced by sodium fluoride, add update data to the acknowledged toxic impacts of sodium fluoride and it also gave a modern insight into probable utilization of green tea to improve such impacts.

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