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Effect of fluoride on brain of albino-rabbit - An experimental study

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Abstract
Background: Fluoride is present in environment in various forms and ingested by man from solid foods, drinking water and inhaled from the air. Out of these, fluoride is present in large quantities in dissolved state in many sources of drinking water producing toxicity in man. Fluoride can cross the blood-brain barrier and it can cause adverse effects on the brain cell architecture, metabolism, enzymes and overall adverse effects on mental functions. Traces of fluoride are essential and beneficial to human being in minute concentrabbitions in preventing dental carries and osteoporosis. However, intake in large quantities produces adverse and toxic effects on our body.
Objective: The present study was designed to investigate the toxic effects (evaluated as histopathological changes) of sodium fluoride on the brain (Cerebral cortex) in albino rabbit.
Materials and methods: Total 6 albino rabbits were used for this study, among them 2 rabbits were taken in the control group (Group A) and 2 rabbits each were taken in both group B and group C who were administered low and high dose of fluoride respectively. After 16 weeks, the brains of each group of the rabbits were studied in respect to their histological change.
Result: Histological changes in the brains (Cerebral cottex) of both Group B and Group C rabbits, following continuous daily exposure to sodium fluoride solutions in two different doses (0.5% solution for Group B and 3% solution for Group C) for 16 weeks of time were studied in detail and compared with those of the controls (Group A).
Conclusion: It is concluded that Sodium Fluoride solution in high doses for prolonged period has a definite adverse effect on the brain parenchyma.

Keywords: Albino- rabbits, Fluorosis, Brain histology, Cerebral cortex damage, Sodium fluoride

Introduction
Fluoride toxicity through drinking water is well-recognized as a global problem. It is probably the first inorganic ion which drew attention of the scientific world for its toxic effects and now it is consumed in high quantities which revealed severe damage to most tissues including primarily the dental, skeletal systems, soft tissues like liver, kidney and brain [1, 2]. With several literatures, it was found that effects of fluoride on the brain and its activity have become the subject of considerable interest in the field of fluoride toxicity [3]. Fluoride cause neuro-degeneration by crossing blood brain barrier [4]. Adverse neurological effects were observed in the brain of humans with exposure to fluoride [3]. With different studies fluoride accumulation was observed in the brain of animals exposed to chronic fluoride intake and this accumulation increased as drinking water fluoride content increased [5]. Human neurological complications such as paralysis of limbs, vertigo, spasticity in extremities and impaired mental IQ were observed with long term intake of high level of fluoride [6]. The cerebral cortex comprises the motor areas, sensory areas and important vital centers.
Our study aimed to investigate the possible pathological changes in rabbit brain. In this study, a histopathological analysis of rabbit's cerebral cortices were done after albino-rabbits were exposed to sodium fluoride solution for a period of 16 weeks in 2 different doses. And data obtained after 16 weeks were documented.

Materials and methods
The present study had been undertaken in the department of Anatomy, S.C.B. Medical College, Cuttack, in collaboration with Department of Pharmacology and Department of

Pathology, S.C.B. Medical College, Cuttack and IMS and SUM Hospital, Bhubaneswar. Ethical clearance from the Institute Ethical committee was obtained for the study. Six (6) healthy, mature, male albino rabbits (Class-Mammalia, Order-Logomorpha, Family-Leporidae, Genus-Oryctolagus, Species-Cuniculus) were selected for the study which weighed between 1.5 and 2.0 Kg. and divided into 3 groups A, B, and C (Fig. 1a-f). Group A of 2 rabbits was the control group whereas Groups B and C were test groups of 2 rabbits in each.

All 3 groups were housed separately where in addition to normal diet; Groups B & C were supplemented with 0.5% and 3% of Sodium fluoride solution orally through feeding tube which provided 5 mg and 30 mg of Sodium fluoride per Kg. body weight respectively. Animals were sacrificed after the end of the experiment i.e. after 16 weeks and all the brains were removed and processed for histopathological study. The tissue slides were stained with Haematoxylin and Eosin and examined under low power microscope.

Results and Discussion

Gross changes in the body and histological changes in the brains of both Group B and Group C rabbits, following continuous daily exposure to sodium fluoride solutions in two different doses (0.5% solution for Group B and 3% solution for Group C) were studied in detail and compared with those of the controls (Group A). Therefore, in the present study, the observations are grouped as: (1) effect on control animals and (2) effect of fluoride on test animals. In Group-B animals the histological findings remained unchanged after the experiment as was observed in case of the controls. In control and in Group B rabbits, the cerebral cortex contained pyramidal neuron of normal shape and size The nucleus was large and centrally placed. The granule cells were present in scattered manner in the neuropil. (Fig. 1).

However, in Group-C animals the histological findings changed remarkably after the end of the experiment which have been studied and tabulated below (Table 1). In cerebral cortex of the Group C rabbits, the pyramidal neurones lost their angular or pyramidal shape and acquired a plump, ovoid, rounded, or characteristic "ballooned" appearance (Fig. 2). The nucleus was displaced to the periphery or axonal base and was shrunken, pyknotic, or absent. Deeply stained atrophied nuclei in some glial cells were visible. At some locations, granule cells showed irregular arrangement with deeply stained, hypertrophied hyperchromatic nuclei. Some glial cells appeared darkly-stained with dot like shrunken nuclei and empty spaces around them.

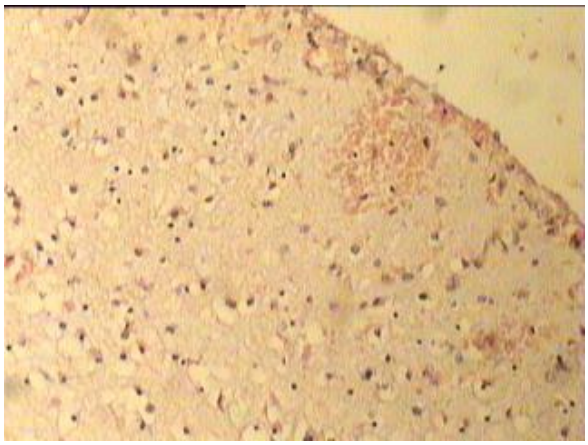

Fig 1: T.S of cerebral cortex of control rabbit showing normal shape and size of pyramidal neuron with dendrites, and granules cells.

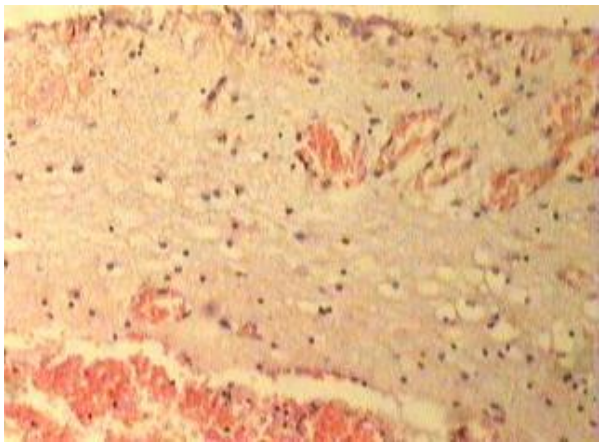

Fig 2: The neurones lost their angular or pyramidal shape and acquired ovoid, rounded, or characteristic "ballooned" appearance. Haemorrhagic congestion of blood vessel are seen.

Table 1: Gross and brain cyto-architecture changes of experimental animals at the end of 16 weeks.

Sl no	Time of sacrifice	Changes in Brain Cytoarchitecture	Blood vessels	Other Gross changes
1	16 Weeks	The neurones lost their angular or pyramidal shape and cquired a plump, ovoid, rounded, or characteristic "ballooned" appearance (Figure 1). The nucleus was displaced to the periphery or axonal base and was shrunken, pyknotic, or absent. Reduction in the number of Purkinje neurones and in some areas even complete loss of neurones. Pyramidal neurones exhibited chromatolysis, Nissl substance was not detectable, and the perikaryon was filled with numerous small colorless vacuoles (Figure 2). Neuroglial cells showed chromatolysis, and in the neuroplasm of these cells, one or two spheroid bodies were present. The nucleus was located at the periphery of the cell (Figure 3).	Highly dilated and congested with extravasation of RBC.	Animals were weak, lethargic and developed paralysis of limbs i.e. spastic paraplegia & quadriplegia.

At some places the neuroplasm and dendrites were filled with black, granular, amorphous material, (Fig. 3). Some neurones showed more advanced disorganization with the retention of disintegrated nuclei and vacuolated neuroplasm along with chromatolysis of neurons ang glial cells (Figure 4). The neuroglial cells (GL) exhibited chromatolysis

(Figure 5) and were hypertrophied. In some neurons a dot-like nucleus, and spheroid or ovoid bodies were present in the neuroplasm. The neurone nucleus was sufficiently enlarged with a shift to the periphery. Some pyramidal neurons showed chromatolysis and were shrunken with vacuolation around them. Highly dilated and congested blood vessel with extravasation of RBC were found in some areas of brain (Fig. 6).

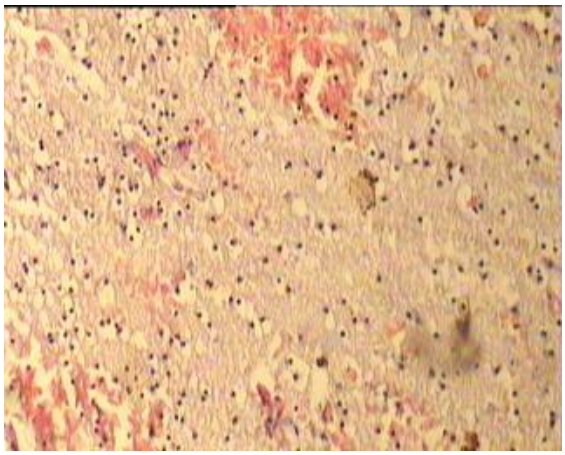

Fig 3: T. S of cerebral cortex showing pyknosis in granule cells and some neurons were seen with constricted, dot like appearance and deeply stained nucleus.

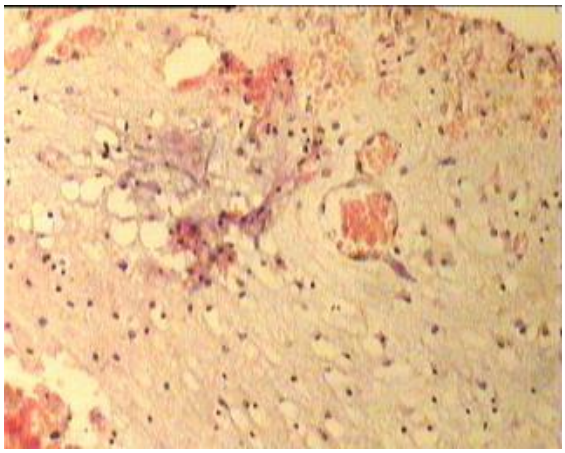

Fig 4: Pyramidal neurones exhibited chromatolysis, Nissl substance was not detectable, and the perikaryon was filled with numerous small colorless vacuoles.

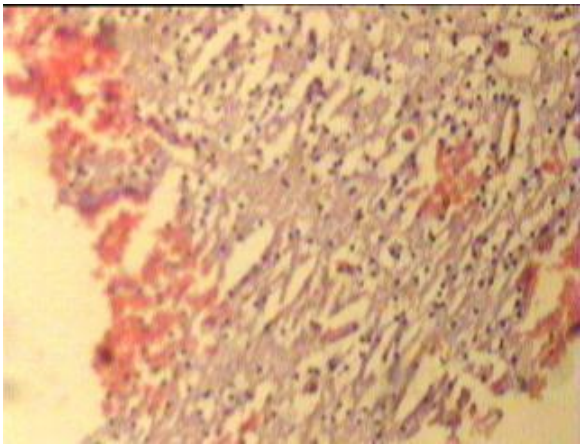

Fig 5: Neuroglial cells showed chromatolysis, and in the neuroplasm of these cells, one or two spheroid bodies were present. The nucleus was located at the periphery of the cell

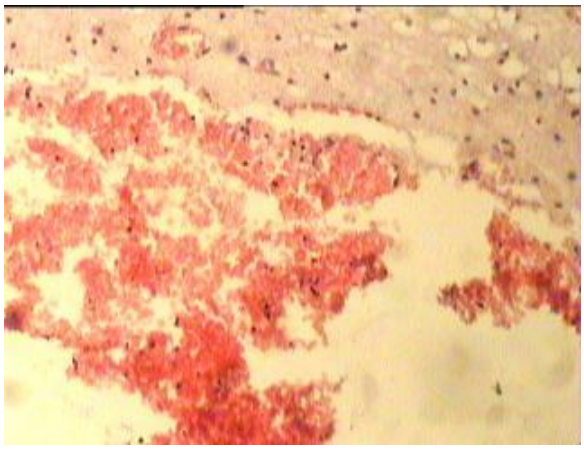

Fig 6: Highly dilated and congested blood vessel with extravasation of RBC.

In the present study, the Group-B rabbits treated with 5mg NaF/kg bw/day showed no abnormalities in the gross features of the body as well as in the cytoarchitecture of the brains. In control rabbit, the cerebral cortex contained pyramidal neuron of normal shape and size the nucleus was large and intensely stained. The granule cells were scattered in the neuropil. Extensive epidemiological and experimental studies have established that the biological responses of animals to fluoride are related to dosage and other factors that influence the animal's physiological and anatomical responses. Evidence that fluoride crossed the blood brain barrier [4] raised the possibility that fluoride could affect the structure and function of the central or peripheral nervous system. The histology of cerebral hemisphere was altered by sodium fluoride [7]. The observations of present work revealed marked alternations in the neuropathology of the cerebral cortex of fluoride treated rabbits. Many granule cells were swollen in shape and size while some appeared darkly stained with dot like shrunken nuclei and empty spaces around them. Some pyramidal neurons showed chromatolysis [8], and were shrunken with vacuolation around them. These results are in accord with the results of [9-12], who documented distinct morphological alterations in the brain including effects on neurons of the cerebrum.

Conclusion

The neurotoxic changes in the brain of our rabbits indicate apoptosis of the neurones and neuroglial cells due to fluoride toxicty. The data suggest that there is a direct action of fluoride in high dosage level upon the nervous tissue, which is responsible for paralysis, seizure, tremors, and sensory deficits and is indicative of brain dysfunction in experimental fluorosis.

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