Histological Study on the Possible Protective Role of Chromium Chloride versus Sodium Fluoride Induced Changes on the Cerebellar Cortex of Adult Male Albino Rat.

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List of abbreviations:

* Analysis Of Variant:	ANOVA
*Central nervous system	CNS
*Chromium chloride	Cr Cl
* Diaminobenzidine tetrahydrochloride:	DAB
*Glial fibrillary acidic protein	GFAP
*Haematoxyline and eosine stain	H&E
*Intelligence Quotient	IQ
* Probability of significance value:	P value
* Rough endoplasmic reticulum	rER
*Sodium fluoride	NaF
* Standard Error of mean	SEM
*Transmission electron microscope	TEM

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Abstract

Fluoride is an essential component for normal bone mineralization and formation of dental enamel. In excessive amounts, it may result in fluorosis. Fluorosis is a slow, progressive degenerative disorder that affects cerebellum as a part of the nervous system.

This study was done to **i**nvestigate the possible toxic effect of low and high doses of sodium fluoride (NaF) on the cerebellar cortex of adult male albino rat for different periods of administration, and to detect the possible protective effect of chromium chloride (Cr Cl).

Sixty-five adult male albino rats were used in this study. They were divided into six groups; each group was subdivided into subgroups **(a and b)** which were treated for one and two months, respectively. **Group 1** (control), **Group II** (treated with daily Cr Cl 300 µg/Kg), **Group III** (oral administration of daily NaF 6 mg/Kg), **Group IV** (oral administration of daily NaF 12mg/ Kg), **Group V** (oral co-administration of daily Cr Cl 300 µg/Kg and NaF 6 mg/Kg), **Group VI** (oral co-administration of daily Cr Cl 300 µg/Kg and NaF 12 mg/Kg). At the end of the experiment, the animals were sacrificed and the cerebella were dissected out and examined by light microscope with H&E, toluidine blue and Glees and Marseland's technique. Immunohistochemical staining was done for glial fibrillary acidic protein (GFAP). The cerebella were also processed to be examined by transmission electron microscope.

The cerebellar cortex of NaF treated rats showed different degrees of neuro-degeneration. Light microscopic

examination showed that the most affected layer was Purkinje cell layer. These degenerative changes were confirmed by statistical results. Increase in positively immunostained glial cells was observed in the molecular and granular layers. In severe toxicity glial cells extended to the Purkinje cell layer. Electron microscopic examination showed ultrastructural changes of Purkinje cell organelles as well as granule cells degeneration. Co-administration of Cr Cl ameliorated these toxic changes in different degrees.

Conclusion: Administration of NaF triggered different toxic changes in the histological architecture of the cerebellar cortex. Long duration of NaF administration induced more significant changes rather than the increase in the administrated dose. It was also observed that Cr Cl could provide partial protection against NaF induced toxicity.

Introduction

Fluoride in minute quantity is an essential element for normal mineralization of bones and formation of dental enamel. On the other hand when fluoride is taken up in excessive amounts, it may cause clinical disturbances in animals and human beings such as fluorosis. There are more than 20 developed and developing nations that are endemic for fluorosis. These are Argentina, USA, Morocco, Algeria, Libya, Egypt, Jordon, Iran, and Iraq **(Shahid et al., 2008).**

Fluoride accumulation was observed in the brain of experimental animals exposed to chronic high-fluoride intake, and this accumulation increased as the drinking water fluoride levels increased **(Shivarajashankar, et al., 2002).**

Further fluoride sources, other than drinking water, are drinks as juice, soda and black tea, tooth paste (1000-1500 ppm), mouth rinse (230-900 ppm), dietary supplements and foods as processed cereals, canned fish and infant formulas **(Bera et al., 2007).** Fluorine is also incorporated in some drug structures to reduce drug metabolism. Numerous drugs contain fluorine including some antipsychotics, human immunodeficiency virus protease inhibitors such as tipranavir, antibiotics such as ofloxacin and trovafloxacin, and anesthetics such as sevoflurane **(Park et al., 2001 and Fisher et al., 2006).**

Sodium fluoride is an extremely toxic substance. Toxicity and even death may occur in children by

swallowing tablets or gels and in adults as in accidents involving fluoridation equipment and filters on dialysis machines **(Connett, 2004).**

Fluorosis caused by excess intake of fluoride is a slow, progressive degenerative disorder known to affect the structure and function of skeletal muscle, brain, and spinal cord **(Trivedi, et al., 2007).** Moreover, long-term intake of high levels of fluoride in humans causes neurological complications such as paralysis of limbs, vertigo, spasticity in extremities, and impaired mental acuity **(Shivarajashankar, et al., 2002).**

Chromium is a mineral that humans require in trace amounts. It is found primarily in two forms which are trivalent and hexavalent. Trivalent (chromium III) is biologically active and found in food. Hexavalent (chromium VI) a toxic form that results from industrial pollution. Chromium is an essential nutrient involved in the regulation of carbohydrate and lipid metabolism. Chromium has now been of interest for its possible connection to various health conditions **(Anderson, 2009).**

Aim of this work:

Aim of this work was to investigate the possible toxic effect of different doses of sodium fluoride (NaF) on the cerebellar cortex of adult male albino rats for different periods of exposure. The possible protective effect of chromium chloride was also investigated.

Review

❖ Fluoride:

Fluoride is the anion (F^-), the reduced form of fluorine. Fluoride is a monovalent ion (−1 charge). Organic and inorganic compounds containing fluorine are called fluorides. The range of fluorine-containing compounds is considerable as fluorine is capable of forming compounds with all the elements except helium and neon. Fluorine-containing compounds range from potent toxins to life-saving pharmaceuticals and from inert materials such as calcium fluoride to the highly reactive sulfur tetrafluoride. **(Greenwood, et al., 1997)**

Peter, (2010) mentioned that the French chemist Henri Moissan prepared elemental fluorine in 1886 by electrolysis. Fluoride was found to be very useful as it could be added to toothpaste as "caries-preventive". On the other hand, he mentioned that fluoride can be very dangerous as it reacts with hydrogen with explosive violence. For this reason, it was used in the United States during World War II for the production of uranium hexafluoride. At elevated temperature, hexafluoride is a gas that may be used for the separation of uranium isotopes. The enriched radioactive uranium was used for the construction of the first atomic bombs which went down on Hiroshima and Nagasaki in 1945. Uranium refining for nuclear energy is still one of the major uses for elemental fluorine.

❖ Sources and uses of fluoride:

The fluoride element is found in the environment and constitutes 0.06-0.09% of the earth's crust. Fluoride is commonly associated with volcanic activity and gases emitted from the earth's crust. It is present in water, foods and air. Thermal water especially those of high pH, are also rich in fluoride. Higher fluoride concentrations found in ground water due to the presence of fluoride bearing minerals. As for food, it has been shown that vegetables and fruits have low fluoride levels (0.1- 0.4 mg/kg). On the other hand, dry tea leaves have significantly high levels of fluoride up to 400 mg/kg. Fluoride is not found naturally in the air in large quantities (0.5 ng /m^3). Fluoride has various uses in many industries including toothpaste, ceramics, and bricks. Toothpaste contains very high concentrations of fluoride up to 1000-1500 mg/kg; however what is accidentally swallowed and ingested may range up to 3.5 mg/day. It has shown that with all human exposure to fluoride that varies from region to region, drinking water is the largest single contributor to daily fluoride intake. **(Abu Zeid, 1998)**

Park, et al., (2001) and Fisher, et al., (2006) mentioned that fluorine is incorporated in some drug structures to reduce drug metabolism. Numerous drugs contain fluorine including some antipsychotics, human immunodeficiency virus (HIV) protease inhibitors such as tipranavir, antibiotics such as ofloxacin and trovafloxacin, and anesthetics such as sevoflurane.

Helal, et al., (2006) stated that Egypt is one of about 21 developed and developing nations that have problems with endemic fluorosis. The main pathway of fluoride (F) exposure is the ingestion of tap water from contaminated ground water sources. The fluoride concentration in industrial waste water samples collected from Abu Zabaal and Dahlia areas around Cairo during six months varied from 1.13 to 7.10 mg/L; significantly exceeding the World Health Organization recommended maximum concentration which is 1 mg F/L.

❖ Effect of different doses of fluoride:

Gessner, et al., (1994) studied the lowest dose at which effect was observed. They found that 16 % of the patients received an estimated dose of less than 1.0 mg/kg body weight of fluoride. They concluded that the lowest estimated dose of fluoride that caused symptoms was 0.3 mg per kilogram.

Whitford, (1996) noted that the probably acute toxic dose of elemental fluoride that could cause serious or life-threatening systemic signs and symptoms was 5 mg/kg body weight. He recommended immediate emergency treatment and hospitalization.

Jean, et al., (2005) found that the lethal dose of sodium fluoride (NaF) for most adult humans was estimated at 6-10 grams which was equivalent to 32 to 64 mg elemental fluoride/kg body weight.

Moreover, epidemiological studies confirmed that chronic toxicity and skeletal fluorosis can be elicited when water concentration of fluoride exceeded 2.5 ppm. So, approximately 60 countries have taken the opportunity to put roughly 1 ppm F into their reservoirs, so water fluoridation is a worldwide phenomenon **(Skinner and Catherine, 2007).**

In addition, **Viswanathan, et al., (2009)** found that increase of fluoride level above 1.33 ppm in drinking water increased the community fluorosis index (CFI) value more than 0.6, an optimum index value above which fluorosis is considered to be a public health problem.

❖ Effect of fluoride toxicity on different body organs:

Whitford, (1994) reported that fluoride is rapidly and extensively absorbed from the gastrointestinal tract. About 80-90% of the ingested amount is absorbed from the gastrointestinal tract depending on the pH of the gastric contents. He stated that absorption is reduced by calcium and certain other cations and by elevated plasma fluoride levels. Factors that acidify the urine increase the retention of fluoride. About 99% of the body burden of fluoride is

associated with calcified tissues. He also observed that fluoride was associated with structural changes in soft tissues and disorders in their function.

Liver as a very active site of metabolism is especially susceptible to fluoride intoxication. Histopathological changes in liver of young albino rabbits following chronic and acute exposure to sodium fluoride were observed by **Shashi and Thapar, (2000).** They noticed hepatocellular necrosis, degenerative changes, hepatic hyperplasia, and extensive vacuolization in hepatocytes and centrilobular necrosis in the liver of the exposed animal. They also found that the central vein and sinusoids of the liver were dilated and engorged with blood with small areas of haemorrhages. These effects were not observed in the control group.

Chinoy, et al., (2001) reported that occupational exposure to organic fluoride induces abnormal menstruation and increase in the frequency of miscarriages. They studied the effects of sodium fluoride on ovary and uterus of female albino mice. Their results showed significant decline of ovarian proteins and increased cholesterol levels in the ovary suggesting altered steroidogenesis. Accumulation of glycogen in uterus was also observed and could be related to affection of carbohydrate metabolism. Treatment by fluoride also caused a hypercholesterolemic effect in serum. These changes were mainly caused by increased tissue burden of fluoride which resulted in increased lipid peroxidation and formation of oxygen free radicals.