



Impact of Sodium Fluoride on Histopathology of Testes In Albino Mice and Its Recovery with Vitamin - C

KEYWORDS

Sodium Fluoride, Mice, Histology, Vitamin – C

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ABSTRACT Increasing in-fertility has become a serious issue. Sodium fluoride is wide spread natural pollutant and large number of the world population is exposed to high doses of fluoride. So it is of special concern to investigate the role of the vitamin- C on effect of sodium fluoride on male reproduction. Male albino mice were treated with 5 ppm of sodium fluoride for 7 and 30 days. After stipulated period testes was isolated and histological work was done. Many changes were observed in the testes of the treated mice like severe degenerative changes in seminiferous tubules, reduced spermatozoa, and necrotic changes in spermatids. The administration of ascorbic acid to NaF-treated mice revealed marked recovery from fluoride toxicity. Histological changes of testes show that fluoride toxicity is reversible and transient with ameliorative effects of ascorbic acid.

INTRODUCTION

Fluorosis is a high risk factor due to excessive ingestion of fluoride. Fluoride is present widely in nature in many forms and its compound for being used extensively. Fluoride enters the body through many sources like drinking water, food, tooth-paste and other dental products, drugs, fumes from industry using fluoride containing salts & hydrofluoric acid and as well as fluoride dust (Shulman & Wells, 1977). The tolerable limit of fluoride in drinking water is 1ppm. In India, the drinking water contains 10 to 25 ppm of fluoride which results in inhibition in the Krebs's cycle (Bogin et al. 1976) as well as induction of muscle atrophy (Susheela and Kharb, 1990), liver toxicity (Saralakumari et al., 1988), and kidney toxicity (Suketa and Terui 1980).

In take of high levels of fluoride was known to cause structural changes of some biological activities, altered activities of enzymes and metabolic lesions in brain (Shivarajashankara et al., 2002). To determine the pathological response of an animal to any toxicant, histology helps to show how these substances cause injury to tissues. There is also evidence of F-induced oxidative stress in the testis and ovary as well as in other organs (Chinoy 2005). Hence the present study is aimed to study the histology of testes in albino mice. The effects of Na F toxicity on mice testis and the influence of vitamin C on various aspects of testicular function after F ingestion were under taken. Vitamin -C, due to its active antioxidant as well as Detoxification properties, is a promising and potent agent in suppressing fluoride toxicity. In addition, it has involved in various metabolic processes.

MATERIALS AND METHODS

Sodium fluoride (99%) was used as a toxicant. Healthy adult Male Wister strain Albino mice of the same age group 70 ± 5 days and weight (30 ± 5 g) were taken from parental stock obtained from veterinary college, Bangalore, and maintained a colony. They were kept in well cleaned and sterilized cages. The mice were maintained at laboratory conditions ($26 \pm 2^\circ\text{C}$; 12hrs light and 12 hrs darkness) with food and water *ad-libitum*.

Experimental design:

The animals were divided into four groups having ten ani-

mals each. All treatments were given orally with a hypodermic syringe attached to an angular needle. The first group of animals with deionized water and the second group of animals with vitamin 'C' (15mg /animal/day) orally by gavage respectively for 30 days were treated as control. Third group of animals were treated with 5ppm of sodium fluoride for 30 days and fourth group of animals were treated with combined dose of 5ppm of sodium fluoride with vitamin 'C' respectively for 30 days (15mg/animal/day). Second & fourth group animals were administered vitamin-C with 48 hours interval. After the stipulated period the tissues from all the groups were isolated for histopathological examination according to Humason, (1972).

Group	Treatment	Duration (7 & 30 days)	
I	Controls treated with Deionized water	30	
II	Treated with De Ionized water + Vitamin C (15 mg/day/animal)	7	30
III&V	Treated with sodium fluoride (5 ppm)	7	30
IV & V1	Treated with combined dose of 5ppm of sodium fluoride and vitamin C	7	30

HISTOLOGY OF TESTES

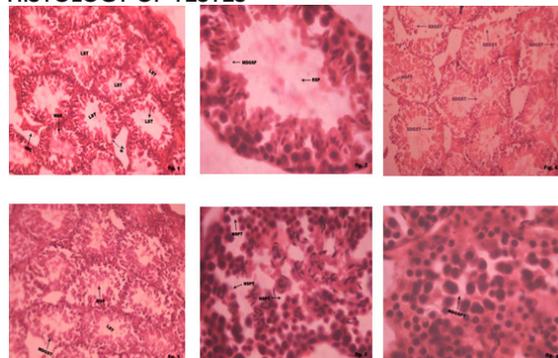


Fig - 1 – Microphotograph of normal structure of testes of

Control mice, showing seminiferous tubules (ST), Interstitial cells (ISC), Mature Spermatozoa (MSP), and Lumen of Seminiferous tubule (LST) H&E staining. 100 ×.

Fig – 2– Microphotograph of 7days exposed testes of mice to sodium fluoride showing moderate degenerative changed in &seminiferous tubule (MDGST) and reduced spermatozoa lumen of seminiferous tubule.(RSP). 400X

Fig – 3 – Microphotograph of 7days exposed testes of mice to sodium fluoride plus vitamin c showing moderate degenerative changed in &seminiferous tubule (MDGST) and reduced spermatozoa lumen of seminiferous tubule.(RSP). 400X

Fig – 4 – Microphotograph of 30days exposed testes of mice to sodium fluoride showing Severe degenerative changes in seminiferous tubule. (SDGST) necrotic changes in spermatids. (NSPT) H&E. - 100X

Fig – 5 – Microphotograph of 30 days of NaF treated mice testis showing Necrotic changes in spermatids (NSPT) .H&E-400X

Fig – 6 – Microphotograph of 30 days exposed testes of mice to sodium fluoride plus vitamin c showing Moderate degenerative changes in spermatids (MDGSPT). H&E - 400X

DISCUSSION:

The present study was aimed to determine the toxic effect of NaF histology in mice reproductive tissue and its recovery with vitamin C.

Histological studies helps to understand the pathological conditions of the mice. In the present investigation, histological alterations was clearly exhibited in the testes of the mice treated with 5ppm concentration of sodium fluoride and vitamin c were represented in figures 1to 6 . In sodium fluoride exposure the testes was much more damaged in 30 days when compared with 7 days. The histology of testis in the present work revealed that the control testis showed normal seminiferous tubules, spermatozoa, and leydig cells lying between seminiferous tubules. The Naf treated mice showed cytoarchitectural changes like moderate and severe degenerative changes in seminiferous tubules, necrotic changes in spermatids and reduced spermatozoa lumen of seminiferous tubules. Whereas in the recovery studies i.e. treatment with Vitamin C showed partial recovery of tissue damage when

compared with Naf treated mice.

In support to our work several authors observed different changes in the testis of mice. Testis histopathology following F treatment revealed loss of spermatozoa, pyknosis, vacuolization, disorganization of germ cells, and atrophic Leydig cells, supporting the view that oxidative stress induced by F affected gonadal functions resulted in severe alterations in the histology of testis which disturbed the process of spermatogenesis (Chinoy et al., 2005). Numerous tubules contained hyper tropic giant cells of different sizes in the lumen which may be generated either due to failure of chromosome replication or cell division causing cell death.

Earlier studies on mice, rats, and rabbits treated with different doses of fluoride for varied durations. (Entissar et al., 2009) have also reported altered histology of testis with structural defects in spermatids and sperms. Sakr and Azabet et al., (2001) reported abnormal seminiferous tubules with many vacuoles, marked reduction in spermatogenic cells, and degenerated Leydigcells in albino rats inhaling pyrethroids. In addition, Manna et al., (2004) found edema between seminiferous tubules, vacuolization, and hyalinization in the tubules of the testes of rats exposed to -cypermethrin. In addition, a decrease in luminal sperm and apparent dilation of tubules, together with oxidative stress, has been associated with testicular damage from NaF in rats. (Ghosh et al., (2002) and Ravisekhar et al., (2011). Similar results were observed when aluminium (3.5mg/kg) was administered to albino mice (Mahita et al.,2011).

Treatment with Vitamin- C has shown clear histological, histo-metric, signs of recovery from NaF exposure-related deteriorations in germ line cells in mice indicating its ameliorative potentials in male sex related toxicology. Similar observations were reported by many authors regarding recovery studies in mice and rat. (Khawaja Ahmad et al., (2012) Hamid Reza Momen et al., (2012)and Madava Rao et al., (2012). Thus it is concluded that, our results indicate that sodium fluoride has a negative influence on testis histological parameters in albino mice. In addition, vitamin C was able to ameliorate the adverse effects of sodium fluoride.

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