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# EFFECT OF ZINC TREATMENT ON EXCESS FLUORIDE TREATED MICE WITH A SPECIAL REFERENCE TO SPERMATOGENIC METAPHASE

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## ABSTRACT

Fluoride (F) toxicity results in reproductive failure in male**.** The present study was undertaken to see the ameliorative effect of Zn supplementation at the level of 2000 ppm in Fluoride (F) induced toxicity in male wister strain mice. The study was carried out by taking 10 animals for each group. One group treated with Fluoride (F) as well as another one treated as Zinc (Zn) supplemented Fluoride (F) treated experimental group and another group served as control. The experiment was carried out for 30 consecutive days. All mice were fed with crushed food pellets ad libitum. Fluoride (F) toxicity arrest the spermatogenesis at the level of metaphase as the metaphase index in fluoride (F) treated group is reduced by 45.86%. Zn supplementation at the level of 2000 ppm failed to increase the Zn content of testis and epididymis significantly and did not change the metaphase index in Fluoride treated mice. However, supplementation of Zn (2000 ppm) can reduce the Fluoride content of bone (femur) ($P < 0.001$) in Fluoride treated group. It has been suggested that excess Zn at the level of 2000 ppm reduced the fluoride load of bone however unable to prevent the fluoride induced reproductive toxicity in male.

**KEYWORDS:** Fluoride, Toxicity, Zinc supplementation, Metaphase Index, Femur.

## INTRODUCTION

Fluoride is one of the potent toxicants' to which humans are exposed. Extensive data on skeletal and dental fluorosis are available (Susheela et al., 1985). However, the effect of fluoride on the structure and metabolism of several soft tissues has been reported recently. Messer et.a1 (1972) reported that low levels of fluoride in food rendered mice infertile, while a high fluoride diet improve their fertility. These reports were contradicted by Tao and Suttie (1976), whose experiments showed that fluoride did not play any essential role in reproduction. Later Kour and Singh (1980) reported that testicular spermatogenesis process was affected in mice administered fluoride at a dose of 500 and 1000 ppm in drinking water. Li et al (1987) claimed that fluoride did not have adverse effects on spermatogenesis or sperm morphology. Earlier, it was reported that ingestion of 10 or 20 mg NaF in mice caused alterations in the histology of reproductive organs, morphology of sperm and induced biochemical changes (Chinoy and Sequeira,1989a; Chinoy and Sequeira,1989b).

The effect of fluoride on male and female fertility has become an area of growing concern. Various studies show that fluoride causes adverse effects on both male and female fertility (Freni, 1994; Chinoy and Narayana, 1994; Susheela and Jethanandini, 1996) Fluoride intake has been linked to lowered human birth rates (Freni, 1994) and decreased testosterone concentrations (Susheela and Jethanandini, 1996). Infertility was found in an area of India where fluorosis is highly endemic (Chinoy and Narayana, 1994). Furthermore, in vitro exposure of human sperm fluoride produced a significant decline in sperm motility (Chinoy and Narayana, 1994a).

NaF has been tested n fertility studies in several species of laboratory animals. Some studies indicate no effects on reproductive function and development (Tao and Suttie, 1976; Messer et al., 1973). Whereas other reveals that exposure to NaF causes reproductive toxic effects (Kour and Singh, 1980; Pati and Bhunya, 1987; Chinoy and Narayana, 1994b).The reported reproductive toxic effects include increased in number of abnormal spermatozoa (Pati and Bhunya, 1987), loss of spermatogenesis (Kour and Singh, 1980), and interference with steroidogenesis (Chinoy and Narayana, 1994b).

Fluoride causes "Zn *deficiency".* Both organic and inorganic fluoride compounds have shown to inhibit Zn containing enzymes, such as carbonic anhydrase (Dugad and Gerig, 1988; Dugad et al., l999; Gelb et al., 1985). Zn depletion follows experimental fluorosis in mice (Kanwar and Singh, 1981). In the liver a significant fall in the levels of Zn was registered. In

rats fed pure spring water (natural F concentration =0.2 ppm or spring water enriched with NaF to result in 0.8, 1.1 or 2.2 ppm F during 180 days, Zn ions were depleted in most tissues.

From the above review it is clearly established that fluoride toxicity has deleterious effect on male reproduction (Chinoy and Sequeira, 1989b; Chinoy, 1995). Fluoride interferes in spermatogenesis and reduces fertility (Chinoy and Sequeira, 1989b; Chinoy, 1995) in rat, ram, mice etc. Data of our laboratory also corroborate above findings in rat. However, it was reported by Kanwar et al. (1981) that fluoride in drinking water causes decrease in Zn content of liver, kidney and bone significantly (Kanwar and Singh ,1981). Krasowska and Włostowski, (1992) and Krasowska and Włostowski, (1996) showed that fluoride intake in rodent leads to histopathological changes in the germinal epithellium of testis associated with Zn deficiency in testis. He also further reported that supplementation of the dietary Zn together with fluoride treatment resulted in complete reversal of the fluoride mediated effect. However, the data in this aspect is not conclusive and insufficient. Chinoy and Sharma, (1998) established that vitamin E and D both have beneficial effects in the reproductive performance of male mice in fluoride toxicity. So it appears that fluoride toxicity and Zn content in testis may have some relationship. Hence, attempts were made to review the situation wheather dietary Zn supplementation can ameliorate the fluoridc induced toxicity in spermatogenesis at the level of metaphase in mice.

## MATERIALS AND METHODS

### Animal selection

Thirty (30) numbers of Balb/c strain male mice were grouped as Control, experimental treated with Fluoride at a dose of 4 mg NaF/100 gm of body weight (wt) and another group treated with same dose of NaF i.e., 4 mg NaF/100 gm of body weight and treared with Zn supplemented (2000 ppm/100 gm of body weight) crushed mice feed (West Bengal Dairy and Poultry Development Corporation Ltd.) ad libitum for thirty (30) days in accordance with 'Institutional Ethical Committee' rules and regulations.

### Grouping of animals

**Control group (C):** Fed with normal crushed food pellets ad libitum for thirty (30) consecutive days.

**Fluoride treated experimental group (F):** Treated with NaF at a dose of 4 mg NaF/100 gm body weight for thirty (30) consecutive days and fed with normal crushed food pellets ad libitum.

**Zinc supplemented fluoride treated experimental group (Zn + F):** Treated with NaF at a dose of 4 mg/100 gm body weight along with Zn ($ZnSO_4$) at a dose of 2000 ppm mixed in crushed food pellets for 30 consecutive days.

During the period of experiment the individual body weight of animals both control and experimental groups were taken. At the end of thirty (30) consecutive days the animals were sacrificed through diethyl ether anesthesia method.

**Estimation of Fluoride in bone (femur)**

The weight of femur was taken and fluoride content in the femur was estimated by colorimetric SPADN method (Parlikar and Mokashi, 2013) and the results were frequently checked by ion selective electrode method (Light and Cappuccino, 1975).

**Estimation of Zn:** The testis and epididymis were digested aseptically in Tri acid (Nitric acid, Perchloric acid, Sulfuric acid; all are GR grade). The volume was made up to 10 ml with double distilled water. Zn was estimated by atomic absorption spectrophotometry (Pulses and oil seed department, Berhampore).

**Study of Metaphase**

Dissected testis was placed 1% tri- Na citrate at 25°C and seminiferous tubules were separated by a needle and kept in 40 minutes within tri-Na citrate solution. Methanol acetic acid (3:1) fixative was added. Afterwards, the materials were placed in 30 % Acetic acid solution for 5 minutes. Cloudy suspension was found. Conventional hypotonic flame dry Giemsa staining technique was adopted to see the state of spermatogonial metaphase (Ghosal and Chakroborty, 1998).

**Statistical analysis**

All the data were analyzed statistically by using student‘t’ test. $P < 0.001$ denotes significant value in our study.

## RESULTS

**Table 1: Average body weight of mice before and after treatment**

| | Control (C) | Fluoride treated (F) (4 mg/100 gm body weight) | Fluoride treated (4 mg/100 gm body weight) along with Zn supplementation 2000 ppm dose with food (Zn+F) |
|---|---|---|---|
| Average body weight (gm) before treatment | 19.5 | 20.5 | 21 |
| Average body weight (gm) after treatment | 26.74 | 27.54 | 31.57 |
| Percentage (%) increase in body weight | 37.13 % | 34.34% | 50.33% |

Body weight of both control and experimental groups were increased during period of experiment.

**Table 2: Estimation of Fluoride content in the bone (femur) of Control, Fluoride treated experimental and Zn supplemented fluoride treated experimental mice**

| | Control (C) | Fluoride treated (F) (4 mg/100 gm body weight) | Fluoride treated (4 mg/100 gm body weight) along with Zn supplementation 2000 ppm dose with food (Zn+F) |
|---|---|---|---|
| **Fluoride content in bone** | **626.50** ± 34.76 ppm | **1599.58** ± 132.64* ppm | **591.03** ± 71.15$^{\#}$ ppm |

C and Zn+F: $P < 0.1$; non significant.

C and F: $P < 0.001$; significant.

Zn + F and F: $P < 0.001$; significant.

**Table 3: Estimation of Zn content (average) in the testis of control, fluoride treated experimental and Zn supplemented fluoride treated experimental mice**

| | Control (C) | Fluoride treated (F) (4 mg/100 gm body weight) | Fluoride treated (4 mg/100 gm body weight) along with Zn supplementation 2000 ppm dose with food (Zn+ F) |
|---|---|---|---|
| Average Zn content in testis (µg/gm) | 15.8 ± 2.2 | 15.02 ±1.3 | 14.3 ± 1.25 |

**Table 4: Estimation of Zn content (average) in the epididymis of control, fluoride treated experimental and Zn supplemented fluoride treated experimental mice**

| | Control (C) | Fluoride treated (F) (4 mg/100 gm body weight) | Fluoride treated (4 mg/100 gm body weight) along with Zn supplementation 2000 ppm dose with food (Zn+ F) |
|---|---|---|---|
| Average Zn content in epididymis (µg/gm) | 29.07 ± 3.25 | 17.03 ±4.35* | 24.4 ± 2.35$^{\#}$ |

*P<0.05 when compared to control group; $^{\#}$P<0.5(NS) when compared to Fluoride treated group.

**Table 5: Percentage (%) of metaphase in testis of control, fluoride treated experimental and Zn supplemented fluoride treated experimental mice**

| | Control (C) | Fluoride treated (F) (4 mg/100 gm body weight) | Fluoride treated (4 mg/100 gm body weight) along with Zn supplementation 2000 ppm dose with food (Zn+ F) |
|---|---|---|---|
| Percentage (%) of metaphase in testis | 0.979 % | 0.53 % | 0.57 % |
| Out of 1000 no. of intact cell no. of metaphase | 10 | 5 | 5 |

**Table 6: Percentage (%) decrease of metaphase in testis in fluoride treated experimental and Zn supplemented fluoride treated experimental mice**

| F | Zn |
|---|---|
| 45.86 % | 41.78 % |

Metaphase count is decreased in both fluoride treated experimental and Zn supplemented fluoride treated experimental group compared to that of control.

## DISCUSSION

It is reported by several workers (Hori et al., 2004) that in fluoride toxicity, homeostasis of Zn metabolism alters. Krasowska and Włostowski, (1992) and Krasowska and Włostowski, (1996) showed that fluoride intake in rodent leads to histopathological changes in germinal epithelium of testis accompanied with Zn deficiency. It appears that Zn deficiency may be

one of the causes of histopathological changes of testis in fluoride toxicity. Zn supplementation can enhance the Zn content of different organs of rat, mice and other species. It is reported that Zn content of the adult testis fails to accumulate Zn from the dietary supplementation at a level of 1000-2000pprn. However, the liver, kidney, bone and hair Zn content increase significantly at that level. So a positive Zn balance is achieved at that supplemented level. Therefore we have used 2000ppm of Zn supplementation in fluoride toxicity to see its curative effect in fluoride toxicity in respect of male reproduction.

The 2000 ppm of Zn supplementation does not have any toxic effect, as in our study we have observed from the Table 1 that the average body weight after the experiment remains unaltered compared to that of the control. However in Zn treated group there is approximately 50% increase in body weight at the end of the experiment. In the fluoride treated group the increase is approximately 34%. So Zn supplementation does not hamper the general body growth. So it appears that the dose selected in our study does not have apparent physiological toxic effect.

Considering the fluoride accumulation in the bone (femur) it is noticed from the Table 2 that there is 29.6% increase in fluoride content in the bone (femur) of fluoride treated (4mg of NaF/100 gm body wt.) group compared to control. However in the Zn supplemented group it has noticed that the increase of fluoride is at the level of 4%, though the data are not statistically significant. It is noticed that in the Zn treated group the accumulation of fluoride in bone is comparatively low. The possible cause is not clear.

In our study, from the Table 3, we observed that in the fluoride treated group, the Zn content of the testis did not alter significantly compared to that of the control. Our data does not comply with the findings of Krasowska and Włostowski, (1992). However, Zn supplementation fails to enhance the Zn content of testis. This finding complies with the findings of Chinoy and Narayana , (1994b). But this level of Zn supplementation enhance the Zn of liver, kidney, bone, hair etc. Therefore, it appears that this supplemented level of Zn can put the mice in positive Zn balance.

From the Table 4, it appears that total epididymal Zn content in a Zn treated group is significantly low compared to that of control. However in the fluoride treated group the Zn content of epididymis is not significant compared to that of control. The low concentration of Zn in the epididymis could be related with the fertility of the male mice which is not studied

in the present circumstances, because sperm motility, sperm viability arid capacitance of sperm depends on Zn level of the epididymis.

It appears that Zn treatment fails to cure the spermatogenic arrest in fluoride toxicity. Our findings does not comply with Krasowska and Włostowski, (1992). It is also observed from the Table 6 that the no. of metaphase per l000 intact cells are almost half compared to control in the fluoride treated as well as Zn supplemented group. Therefore it appears that spermatogenic arrest in fluoride toxicity in part due to arrest of metaphase. However Zn supplementation does not ameliorate the spermatogenic arrest at the level of metaphase.

In conclusion we can say that fluoride toxicity arrest the spermatogenesis at the level of metaphase as the metaphase index in fluoride treated group is reduced by 45.86% (Table 6). Zn supplementation did not have any curative effect to overcome the fluoride toxicity in respect of male reproduction. However, supplementation of Zn (2000 ppm) can reduce the Fluoride load of the body.

**Conflict of interest**

The authors declared no conflicts of interest. This research received no specific grant from any funding agency in the public or commercial.

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