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Teratogenicity of sodium fluoride on newly born rats

Abdelalim A. Gadallah^{1,2}¹Zoology Dept., Faculty of Science, Mansoura University, Egypt²Preparatory Year Deanship, Jazan University, Kingdom of Saudi Arabia(Corresponding author e.mail:abdelalim@gmail.com)

Abstract

Fluoride (F) is widely used to sterile drinking water against bacterial infection as well as for normal cleaning of teeth. Although intake of low doses of fluoride is required to prevent dental caries, increased uptake for long time injured bone and soft tissues causes fluorosis (Susheela, 1999). The present study aims to illustrate the teratogenicity and histopathological alterations of fluoride in maternal liver, kidney and thyroid glands. Twenty virgin female and male albino rats of Wistar strain at ratio of 2 female/ 1male were kept under good ventilation with controlled conditions and excess food and water were supplied *ad libitum*. Pregnant rats were arranged into two groups (n= 6) including, control and fluoride-intoxicated group. Body weight, size and crown rump length of newly born rats were determined. The offspring 1-day old were sacrificed by light anesthesia with diethyl ether and immediately fixed in 10% formal saline. Alizarin red S preparation of both control, and experimental groups were made and the incidences of deformed bones were recorded. Histological preparations of maternal liver, kidney and thyroid glands were made and examined under bright field light microscopy. Experimental group exhibited disruption of the normal integrity of hepatic lobules with prominent centrilobular necrosis and dilatation of blood sinusoids. Perivascular leukocytic cell infiltration was remarked with bile duct obliteration. Also, peritubular inflammatory cellular infiltration associated with degeneration of renal tubular lining epithelial cells and reduction of their tubular lumina were also detected. Degeneration of the thyroid follicles with marked reduction and vacuolation of colloid. Few numbers of the thyroid follicles exhibited exfoliation of their lining cells within their follicular lumina. Inter-follicular hemorrhage and congested blood vessels were remarked. Fluoride-intoxication showed abortion of one /6 mothers. There were numerical decreases of offspring of fluoride-intoxicated mother. Increase average of congenital malformations was observed.

Keywords: Fluoride-toxicity, liver, thyroid gland, kidney, mother, offspring

1 Introduction

Fluoride (F) is a widely used to sterile drinking water against bacterial infection as well as for normal cleaning of teeth. Although intake of low doses of fluoride is required to prevent dental caries, increased uptake for long time injured bone and soft tissues causes' fluorosis (Susheela, 1999). Also, Fluoride is a highly reactive inorganic mineral and forms numerous inorganic compounds. It is widely used in manufacture of wire and cable insulations, pipe lining, rodenticides, refrigerants, aerosol propellants, fertilizer, and electronic ceramics (Pratusha *et al.*, 2011). On the other hand, exposure to high concentration of F was found to interfere with dysfunction of teeth and bones. F was found to induce induced apoptosis of epithelial lung cells (Thrane *et al.*, 2000). F intoxications were reported in many organs such as heart, liver, kidneys, gastrointestinal tract, lungs, brain and blood (Perumal *et al.*, 2013). Occupational hazards of fetotoxicity of fluoride were reported since water fluoridation was initiated in the 1940s. Tadpole exposed to sodium fluoride was found to develop reduction in the head-tail lengths and dysfunction of the neuromuscular system (Goh and Neff, 2003). *In vitro* studies of fluoride toxicities on limb bud cells of 13-day rat (6.8 micrograms/ml) and 12-day mouse (7.3 micrograms/ml) for 5 days were found to inhibit cell proliferation and differentiation (Zhang and Wu, 1998). Expose rats (Gupta *et al.*, 2007; Kumar *et al.*, 2010) and mice (Sun *et al.*, 2011) to fluoride in drinking water were found to increase the incidence of infertility and reduce sperm counts. There is a little of work concerned maternal fluoride toxicities.

The present study aims to illustrate the teratogenicity and histopathological alterations of fluoride in maternal liver, kidney and thyroid glands.

2 Materials and Methods

Twenty virgin female and male albino rats of Wistar strain (body weight: 145 ± 3.1 g) at ratio of 2 female/1 male were provided from Hellwan Breeding farm (Ministry of Health, Egypt). They were kept under good ventilation with controlled conditions (12 hr light/ dark cycle; 22–24 °C) and excess food and water were supplied *ad libitum*. Pregnancy was carried out by mating fertile male with virgin female for overnight and examination of vaginal smears on the next morning to determine the onset of gestation. Pregnant rats were arranged into two groups (n= 6) including, control and fluoride-intoxicated group (20mg/kg body weight in tap water, dosed orally from 6th day of gestation till parturition). The animals were sacrificed at parturition according to the bioethics of Mansoura University Committee. Body weight, size and crown rump length of newly born rats were determined. The offspring 1-day old were sacrificed by overdose of chloroform and immediately fixed in 10% formal saline. Skeleton preparation of both control, and experimental groups were made by treatment with 2% potassium hydroxide until ossified areas were clearly visible through the soft tissue and stained by the alizarin red "S" method. The incidences of deformed bones were recorded. Maternal liver, kidney and thyroid glands were incised and fixed in 10% formal saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and mounted in molten paraplast 58-62°C. Five microns histological sections were cut and stained with hematoxylin and eosin and examined under bright field light microscopy.

Biostatistics: The significance test for differences between the control and fluoride-intoxicated group was determined. P value of <0.05 means significant.

3 Results

Maternal liver, kidney and thyroid gland:

Liver: Control liver possessed normal hepatic strands arranged around the central vein with thin blood sinusoids and fine arrangement of Kupffer cells (Fig.1A). Experimental group received daily oral doses of fluoride (20mg/kg body weight) from 6th day of gestation till parturition exhibited disruption of the normal integrity of hepatic lobules with prominent centrilobular necrosis and dilatation of blood sinusoids. Perivascular leukocytic cell infiltration was remarked with bile duct obliteration. Granulomatous lesions were detected (Fig.1 A1&A2).

Kidney: Normal kidney showing normal structure of glomeruli with intact Bowman's capsule and renal tubules lined with epithelium (Fig.1 B). Fluoride intoxication exhibited peritubular inflammatory cellular infiltration associated with degeneration of tubular lining epithelial cells and reduction of their tubular lumina. Glomerulonephritis characterized by reduced Bowman's space were observed in many of the nephron. Peri-glomerular round cell infiltration was also detected (Fig.1 B1&B2).

Thyroid gland: Control exhibited the presence of follicles of varying sizes outlined by cubical follicular cells with rounded vesicular nuclei. Their lumina were enclosed by homogenous eosinophilic colloid material. The inter-follicular cells and blood capillaries occupied the space

between thyroid follicles. The follicles were separated by thin collagen fibers (Fig. 1C). In experimentally group intoxicated with fluoride, there was a detected degeneration of the follicles with vacuolation and reduction of colloid. Few numbers of the thyroid follicles exhibited exfoliation of their lining cells within their follicular lumina. Inter-follicular haemorrhage and congested blood vessels were observed. The follicular epithelium appeared either vacuolated or entirely degenerated (Fig. 1 C1&C2).

Effects on pregnant:

Fluoride-intoxication showed abortion of one /6 mothers. There were numerical decreases of their offspring. Increase average of congenital malformations was observed. Most of the malformations were restricted mainly in fore & hind limb deformation, reduced neck region and kyphosis. Missing ossification of distal phalanx of fore & hind limb and caudal vertebrae. Decreased body weight, size and crown-rump length were also observed (Table 1 & Figs.2 &3).

4 Discussion

Fluoride-intoxication represents one of the important public health problems. Oral intake of sodium fluoride (20mg/kg body weight) from 6th day of gestation till parturition exerted histopathological lesions in liver, kidney and thyroid gland of mother rats. Nephrotoxicities manifested by tubular necrosis and glomerulonephritis, meanwhile centrilobular necrosis and granulomatous lesions were mainly detected liver. Massive atrophy of thyroid follicles with almost loss of their thyroglobulin and degeneration of follicle epithelium were remarked

Similar studies explained the fluoride associated hepatotoxicity (Shashi and Thapar, 2000; Dabrowski et al., 2006; Shashi and Thapar, 2008 ; Atmaca *et al.*, 2014), nephrotoxicity (Kassabi *et al.*, 1981; Kassabi and Braun, 1985; Nabavi *et al.*, 2012) and thyroiditis (Leite Ade *et al.*, 2007; Wang *et al.*, 2009).

The observed damage of hepatocytes, renal cells and follicle epithelial cells supported the findings of Godfrey and Watson (1988) who reported apparent inhibition of the synthesis of DNA, protein and cell replication of rat neuronal cells post-fluoride intoxication.

The observed organs dysfunction were reflected on the development of offspring maternally intoxicated with fluoride. The main congenital malformations explained by decreased body weight, kyphotic body, deformation of fore-&hind limb, kinky tail and reduction of neck region. The overall retarded body growth was assessed by delayed ossification of phalanx and caudal vertebrae and comparatively decreased ossified limb regions and flat bones of skull.

Similar growth deformities of fetuses and increased rates of resorption (Guna sherlin *et al.*, 1999) and skeletal malformations were reported in rat fetuses maternally intoxicated with sodium fluoride (Collins *et al.*, 1995).

Fluoride- intoxication was found to result from absorption of its soluble inorganic components via gastrointestinal tracts and converted to hydrogen fluoride by the acidic environment of the stomach (Whitford and Pashley, 1984) and distributed rapidly by the blood

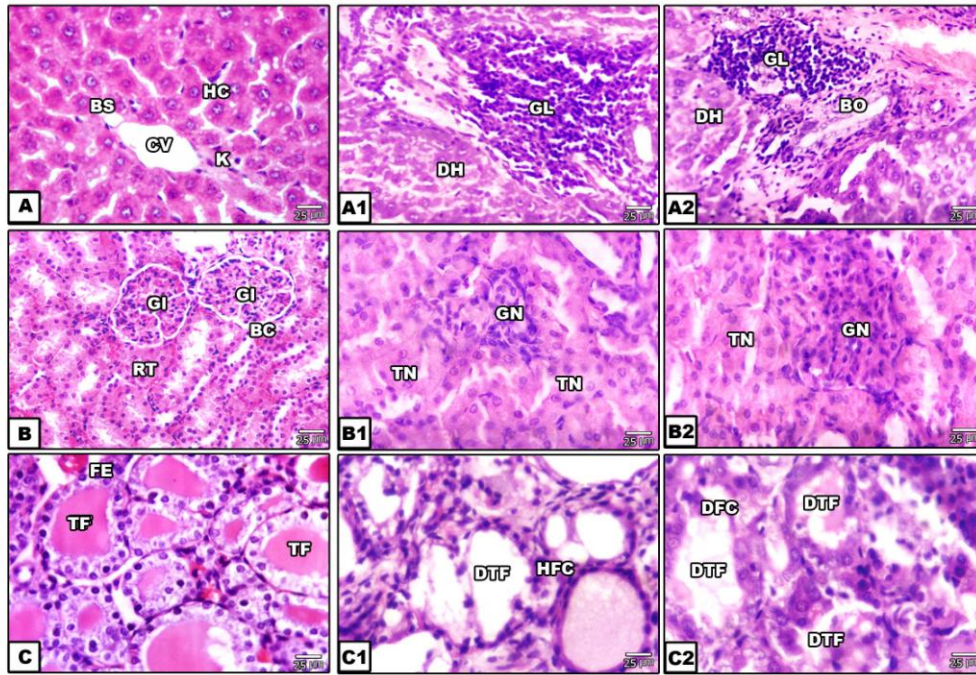


Fig.1. Photomicrographs of liver (A-A2), kidney (B-B2) and thyroid gland (C-C2) of mother rats. A-C. Control. A&A1, B&B1 , C&C1. Fluoride-intoxicated. Note normal pattern structure of hepatic cord and hepatocyte (A), normal renal tubules lined with epithelium and intact glomeruli with Bowmans capsule (B) and regular pattern of thyroid follicles containing thyroglobulin within their lumina (C).A1&A2. Fluoride-intoxicated hepatic tissues showing granulomatous lesions and bile duct obliterations. B1&B2. Fluoride intoxicated kidney showing glomerulo-nephrosis and tubular necrosis. C1&C2. Fluoride-intoxicated thyroid gland showing degenerated epithelium lining the follicles and missing thyroglobulin. **H&E X400**

Abbreviations;BC, Bowman capsule; BO, Bile duct obliteration; BS, Blood sinusoids; CV, central vein;DH degenerated hepatocyte.; DFC, degenerated follicle cells; DTF,degenerated thyroid follicle;FE, Follicle epithelium; GL,Glomeruli ; GL, Granulomatous lesions; GN, Glomerulo-nephritis;HC, Hepatic cord; HFC, , Hemorrhagic follicle cells; RT,Renal tubule; TF,Thyroid follicle; TN, Tubulo-necrosis.

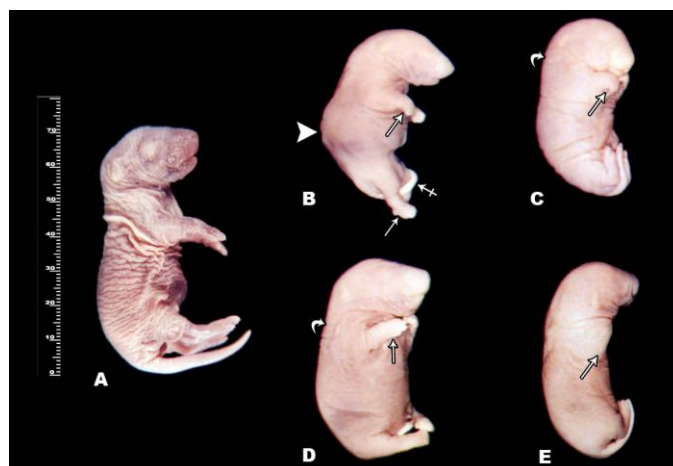


Fig.2. lateral view of gross morphology of newly born 1 day-old of control (A) and maternally intoxicated with fluoride (B-E). B-Showing kyphotic body, malformed hind limb and kinky tail. C. Showing missing neck region and deformities of fore- and hind limb and kinky tail. D. Showing mucinous body and kinky tail. E. Showing deformities of fore & hind limb and kinky tail.

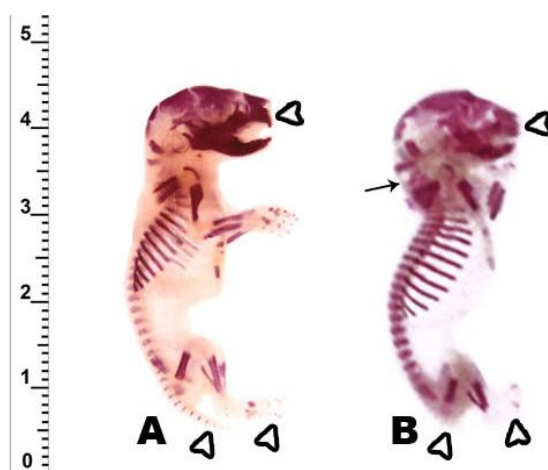


Fig.3. lateral view alizarin preparation of skeleton of newly born 1 day-old of control (A) and maternally intoxicated with fluoride (B). Arrow head indicated delayed bone formation of nasal , sacral and distal phalanges of hind limb.

Table 1. Teratogenicity of rat newly born maternally treated with fluoride from 6th day of gestation till parturition.

	Control	Fluoride-treatment
Total No. of mother	6	6
Aborted mothers	-	1
Total No. of mother undergoing pregnancy	6	5(83.3%)
Total number of newborn	49	34 (69.4%)
Mean number/dam	8.2	6.8(82.9%)
Superficial haematoma	-	4 (11.8%)
Malformed fore limb	-	7 (20.6%)
Malformed hind limb	-	6 (17.6%)
Reduced neck region	-	5 (14.7%)
Kyphotic body	-	8(23.5%)
Deformed neck region	-	5 (14.7%)
Missing Caudal ossification	-	12(35.4%)
Mean body weight (gm)	5.6±0.2	4.2 ±0.3
Mean body size (cm) ³	5.7±0.6	4.7±0.4
Crown-rump length (mm)	45.3±2.5	38.4±3.7

Each result represent the mean±SE (n=10). The data is significant at P< 0.05.

circulation to the intra-and extracellular tissues as well as accumulated in bone and renal tissues, exerted its cytotoxicity (Taves *et al.*, 1983).

The observed congenital malformation may be attributed to transplacental passage of fluoride (Malhotra *et al.*, 1993; Opydo-Szymaczek and Borysewicz-Lewicka, 2007) and incorporation into foetal tissues retarding bone differentiation. Also, the observed hepatitis may be associated with increased 7-dehydrocholesterol reductase hepatic gene expression, leading to depletion of 25-OH vitamin D serum levels. These were associated with high active transforming growth factor-beta (TGF-β) serum levels, impairing osteoblast function in vitro (Nussler *et al.*, 2014).Cirrhotic liver was found to be associated with reduction of tibial bone. volume leading to osteoporosis (Nakano *et al.*,1996).

In addition, thyroid hormone (T3) is important for postnatal skeletal growth via binding to nuclear receptors, TRs including TRα1 and TRβ1, which are important for endochondral bone formation by the cell types

chondrocytes and osteoblasts (Desjardin *et al.*, 2014; Tuchendler and Bolanowski, 2014).Expose stage 55 premetamorphic *Xenopus* larvae to sodium fluoride intoxication at 10 mmol/L led to decrease the expression of the pro-osteoclastogenic factor RANKL and increase the expression of the anti-osteoclastogenic factor osteoprotegerin. These led to marked alterations of osteoblast and osteoclast differentiation, as well as on the expression of osteoblast products, including MMP1 and collagen (Nair *et al.*, 2011). These may be associated with altered ossification in newly born maternally intoxicated with fluoride.

Finally the author concluded that be avoid of dental team as a result of maternal intoxication.

5 References

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