Indian J.Sci.Res. 4(1) : 87-92, 2013
ISSN : 0976-2876 (Print)
ISSN : 2250-0138 (Online)

HISTOPATHOLOGY OF SPLEEN OF RAT (*Rattus norvegicus*) FED ON DOSES OF SODIUM FLUORIDE

ARBIND KUMAR[a1] AND SAPNA KUMARI[b]

Cell Biology and Toxicology Laboratory, P.G. Department of Zoology, Patna University, Patna, India
[a]E-mail: arbindkr7@rediffmail.com
[b]E-mail : sapna501@yahoo.co.in

ABSTRACT

Water is indispensable and one of the precious natural resource of this planet. Ground water is an important source of water supply throughout the world. Fluoride poisoning arises from drinking fluoride rich ground water. Our present experiment was designed to study the efficacy of NaF on the splenocytes and its role in immunogenic response. The albino rats were taken for the experiment and randomly assigned into three groups of each of eight animals. First group and the second group were fed with lower (25mg/kg-bw/day) and higher (50mg/kg-bw/day) doses of NaF for 60 days and the third group rats were kept as control. Later on the animals were killed by cervical dislocation and spleens were dissected out and processed for light microscopy and scanning electron microscopy. In the light microscope it was observed that fluoride causes shrinkage in the white pulp nodule and degeneration of red pulp. Scanning electron microscope also revealed the degeneration of many splenocytes in the fluoride treated group. The changes were more prominent in the rats treated with the higher dose of NaF. The finding suggests that NaF may play a key role in the activity of Ca^{2+} ATPase and adversely affect the cell-cell and cell-matrix adhesion of splenocytes. It was concluded that NaF may adversely affect the structure and function of the spleen particularly due to the apoptosis of splenocytes and degeneration of red pulp in the rat leading to the reduced immunogenic response.

KEYWORDS : Fluoride, Spleen, Splenocytes, Ca^{2+} ATPase, Apoptosis, Immunogenic Response.

Fluoride poisoning arises from drinking fluoride-rich ground water. Excess intake of fluoride results in mainly three forms of fluorosis namely : Dental fluorosis (Dean and Elvove, 1935). Skeletal fluorosis (Mithal et al., 1993; Gupta et al., 1993; Wang et al., 1994) and Non-skeletal fluorosis (RGNDWN,1993). Morphological changes in the spleen of Balb-C mice was reported earlier when the animals were subjected to 10 mg/kg-body weight(bw) and 50mg/kg-bw NaF (Machalinska et al. ; 2002). In an another experiment female Wistar rats when fed on 0.5 and 5mg NaF for 3 months showed the decrease in the lymphoid tissue mass of the spleen (Bely, 2000). Sheep, Awasi breed upon administration of 1500 mg/kg fluoride (hexafluorosilicate) and 2000mg/kg fluoride died after 3hrs and 2.5hrs respectively. Post-mortem examination of the sheep showed hemorrhages occurred on the spleen (Karen et al., 2001)

It has been earlier reported NaF induces renal apoptosis, reduces the cell number of G(2)/M period in cell cycle and decrease the relative of DNA significantly (Yu et al., 2002). Also NaF induces apoptosis in rat brain (Chen et al., 2002), liver (Wang et al., 2004) and testis (Sun et al., 2011) Recently, it has been reported that a higher intake of 800 and 1200 mg F/kg diet inhibited the development of the spleen by inducing lymphocyte apoptosis in young chickens (Chen et al., 2009). Our present experiment is designed to study the efficacy of NaF on the splenocytes and its role in immunogenic response.

MATERIALS AND METHODS

The same protocol was followed in the present investigation as in our previous experiment. Twenty four albino rats, Rattus norvegicus of 3 months old having an average weight of 110g were procured from the Animal House of Banaras Hindu University, Varanasi. The rats were housed in cages under the identical laboratory conditions (12hr/12hr light-dark and ambient temperature 26±2°C) in the colony room at PG Department of Zoology, Patna University, Patna. A standard animal diet and drinking water (fluoride content less than 0.5ppm) were provided *ad libitum*. Prior to the dose treatment the animals were acclimatized for 15 days.

The rats were randomly assigned to three groups each of 8 animals. The doses of sodium fluoride (E.Merck [India] Limited, Mumbai) at 25mg/kg bw/day were given to first group of animals orally as solution in distilled water. The second group of animals was treated with the higher dose of NaF (50mg/kg/bw/day).Third group was paired fed, but not administered sodium fluoride; it was kept as the control. The animals were killed by cervical dislocation

[1]Corresponding author

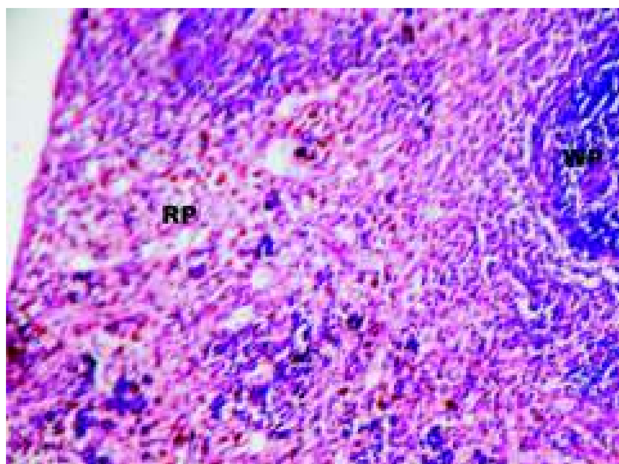

Figure 1 : Photomicrograph of Control Rat Spleen Showing Distinct Red Pulp (RP) And White Pulp (WP). (H-E stain) X 100

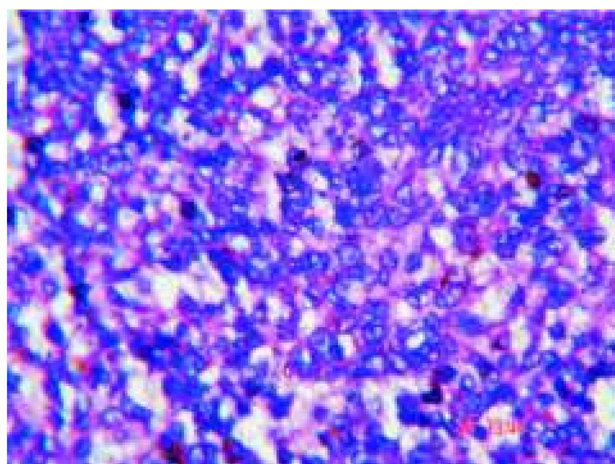

Figure 2 : Photomicrograph Of Control Rat Spleen Showing Normal Morphology Of Red Pulp With Splenic Cords. (H-E stain) X 100

after 60 days treatment along with the controls. The abdominal cavity was opened and spleens were dissected out and washed briefly in normal saline solution. The organ was cut into pieces and fixed in Carnoy's fixative for 3 hours for light microscopy. Some small pieces of the tissue, after washing in cold (4°C) 0.1M phosphate buffer (pH 7.4) were fixed in 2.5% glutraldehyde and 2% paraformaldehyde buffer for six hours at 4°C temperature for electron microscopy.

Light Microscopy

The tissue pieces were washed well after fixation and dehydrated through a graded series of alcohol, cleared in xylene and embedded in paraffin wax. 5µm thick sections were cut and stained with standard Hematoxylin-Eosin. Microscopic slides were observed under light microscope and subsequently photographed.

Scanning Electron Microscopy

Washing the specimens in 0.1M phosphate buffer, post fixing in 1% osmium tetra oxide for two hours and again washing in phosphate buffer, the specimens were dehydrated through ascending grades of acetone. The tissues were further dehydrated, dried at critical point for one hour followed by sputter coating with gold and were examined under the scanning electron microscope (LEO 435 VP) operated at 15 kV.

RESULTS

Light Microscopic Observation

The animals of control group showed the normal morphology of spleen having distintict red pulp and white pulp (Figure, 1). The red pulp shows many cells and splenic cords (Figure, 2). The fluoride treated animals showed the increased red pulp with many infiltrated lymphocytes and decreased white pulp. There was shrinkage in the white pulp nodule. The rats treated with higher dose of fluoride showed less eosinophilic pale staining regions indicating degeneration of red pulp (Figure, 3). The meshwork of

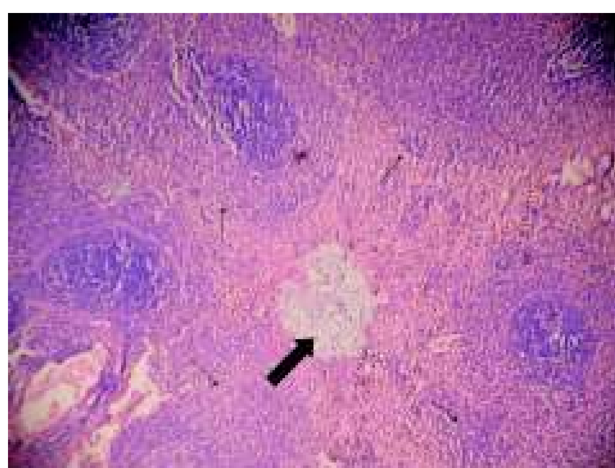

Figure 3 : Photomicrograph of Sodium Fluoride Treated Rat Spleen With A High Dose (50mg/kg-body Weight) Showing Degenerated Area (bold Arrow) In The Red Pulp And Infiltered Lymphocytes (arrows). (H-E stain) X 40

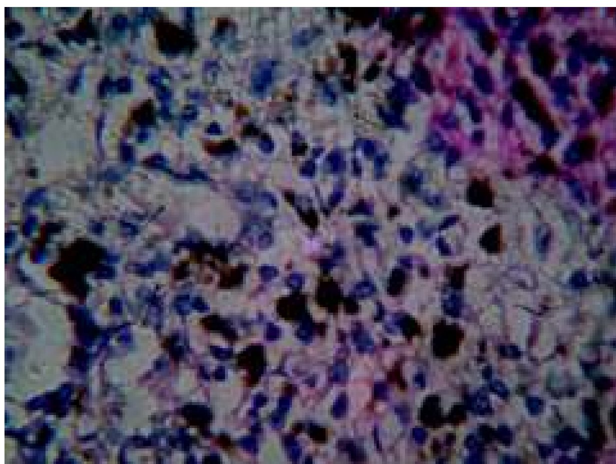

Figure 4 : Photomicrograph of Sodium Fluoride Treated Rat Spleen With A High Dose (50mg/kg-body Weight) Showing Degenerated Splenic Cords (arrow) And Many Apoptotic Cells In The Red Pulp. (H-E stain) X 400

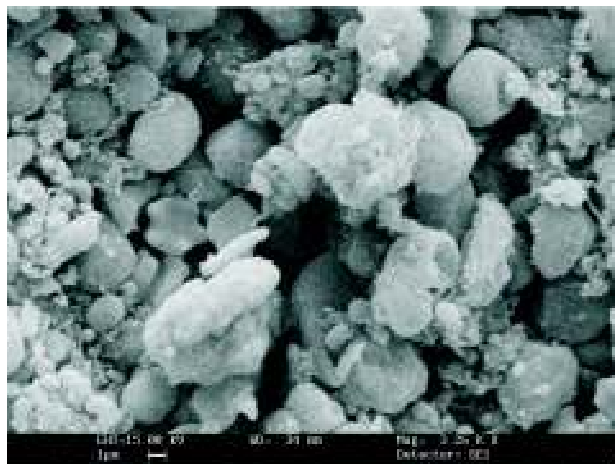

Figure 6 : Scanning Electron Micrograph of Control Rat Showing Normal Morphology of Lymphocyte, Red Blood Cells, Neutrophils, Macrophages And Platelets. X 3260

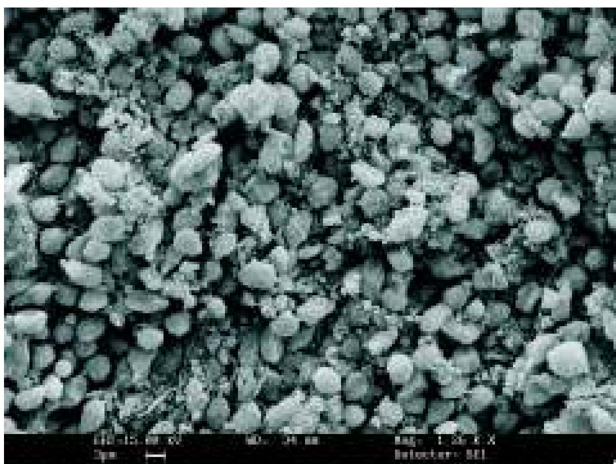

Figure 5 : Scanning Electron Micrograph of Control Rat Showing Normal Morphology of Lymphocyte, Red Blood Cells, Neutrophils, Macrophages And Platelets. X 1260

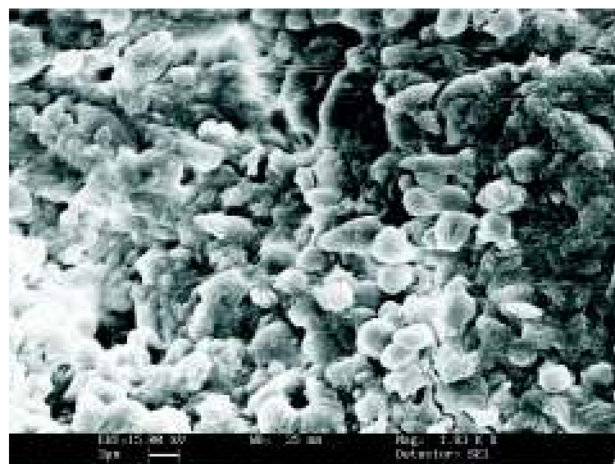

Figure 7 : Scanning electron micrograph of sodium fluoride treated rat with low dose showing deformed shapes and sizes of cells getting adhered to each other. X 1830

reticular fibers in the splenic cords was also seen degenerated (Figure, 4). No such changes were observed in the spleen of rats treated with lower dose of fluoride.

Scanning Electron Microscopic Observation

The control group animals showed cells with normal shape. Red blood cells, neutrophils, macrophages, lymphocytes and plateletes were clearly visible with their normal structure (Figure, 5, 6).

In contrast to the control group the animals treated with the lower dose of fluoride showed many cells with deformed shapes and sizes. The degenerating cells were seen adhered to each other giving a condensed appearance at many places (Figure, 7, 8). The abnormal red blood cells with finger like protrudernce were seen in large numbers (Figure, 8).

The animals treated with higher dose of fluoride showed more prominent degenerative changes. The shape of the cells was drastically changed due to which the neutrophils, macrophages and lymphocytes were imperceptible. (Figure, 9).

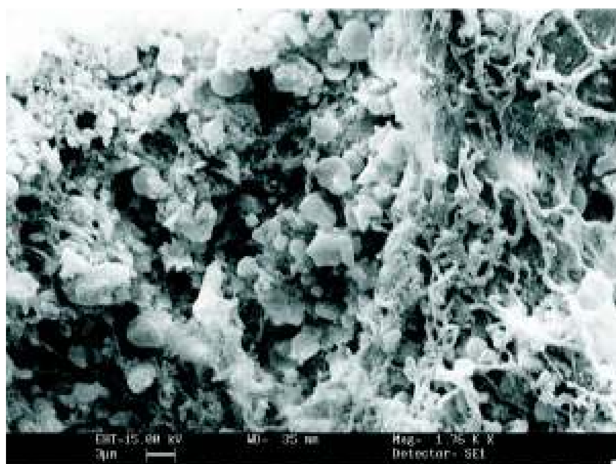

Figure 8 : Scanning Electron Micrograph of Sodium Fluoride Treated Rat With High Dose Showing Abnormal Red Blood Cells With Protuberance On Its Surface. X 1760

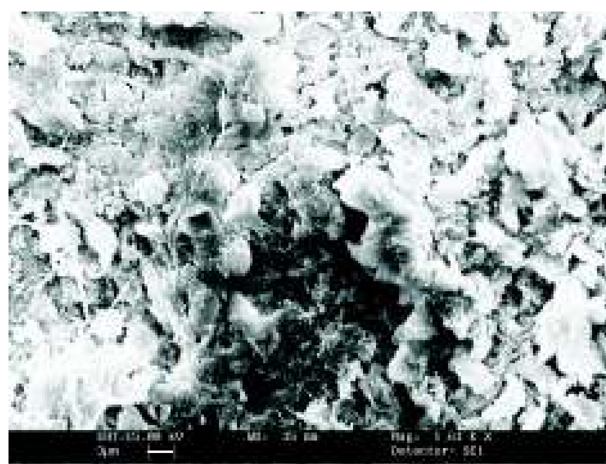

Figure-9. Scanning Electron Micrograph of Sodium Fluoride Treated Rat With High Dose Showing Prominent Degenerative Changes. X 1610

DISCUSSION

It is well known that fluorides have a disruptive effect on various tissues in the body (Takamorim, 1962 ; Vilber et al., 1990 ; Yoshisa, 1959). One of the recent study reported that fluoride induces apoptosis in spleen (Chen et al. ; 2009) .Our present finding that there is decrease in white pulp with concomitant increase in red pulp infiltrated by lymphocytes is in agreement with other findings (Machalinska et al., 2002 ; Podder et al., 2010). The eosinophilic pale staining area found in the red pulp may be due to the apoptosis of the splenocytes. It may also be possible that the fluoride may have cause the degeneration of the extracellular matrix to which the splenocytes have attached. In one of the findings it has been reported that low dose of sodium fluoride causes significantly increased activity of an enzyme Ca^{+2} ATPase and high dose of sodium fluoride causes its inhibition (Hui et al., 2007). Also the increased amount of intracellular free calcium due to sodium fluoride plays a key role on the mechanism of renal injury in fluorosis (Hui et al., 2007). Our proposition is that NaF may play a key role in the activity of Ca^{+2} ATPase which in turn may adversely affect the cell-cell adhesion and cell-matrix adhesion thereby causing the lymphocytes to spread and infitrate in the red pulp from white pulp as observed during the present study. Earlier it has been reported that sodium fluoride induces podosome formation in endothelial cells (Tatin et al., 2010). Podosomes are actin-based structures endowed with adhesion and matrix-degradation functions. It is possible that due to the formation of podosomes in the splenocytes of the red pulp, the matrix had undergone degradation resulting into signaling to the cells adhered with it and causing amplification of cascade of reactions leading to apoptosis.

Light microscopic and scanning electron microscopic observations during the present investigation revealed the degeneration of reticular cells and reticular fibers. It may be possible that the fibers have undergone degeneration due to the fluoride toxicity which in turn may adversely affect the function of resident cells in the splenic cords.

It is previously reported that red cells from humans exposed chronically to toxic levels of fluoride through drinking water showed significant increase in lipid peroxidation and membranous cholesterol and phospholipids there by causing red cell membrane alterations (Kumari and Rao, 1990). In the present investigation scanning electron microscopy has revealed that the normal structure of red blood cells is altered giving finger like protruberence on its surface due to the fluoride toxicity. It may be possible that the fluoride may induce alterations in the biochemical compositions of red cell membrane by generating free radicals and thereby forming abnormal erythrocytes. In one of the findings it has been reported that Rho family GTPases Rho1 and Rac1 regulates

endothelial cells permeability (Wójciak-Stothard et. al., 2001). It is possible that sodium fluoride may play a key role in regulating the genetic expression of Rho family GTPase thereby altering the red blood cells permeability and inducing the formation of abnormal shape of erythrocytes.

In conclusion, sodium fluoride adversely affects the structure and function of the spleen particularly due to apoptosis of splenocytes and degeneration of red pulp in rat. This may result in reduced immunogenic response which will have a direct bearing on the human beings living in endemic areas of fluorosis.

REFERENCES

Bely M., 2000. Lymphoid depletion of spleen due to experimental fluorosis in rats. Fluoride, **33**(1):S1-S2. XXIIIrd ISFR Conference abstract, Szczecin, Poland.

Chen J., Chen X., Yang K., Xia T. and Xie H., 2002. Studies on DNA damage and apoptosis in rat brain induced by fluoride. Zhonghua Yu Fang Yi Xue Za Zhi., **36**(4): 222-224.

Chen T., Cui Y., Bai C., Gong T., Peng Xi. and Cui H., 2009. Increased apoptotic lymphocyte population in the spleen of young chickens fed diet in high fluorine. Fuoride, **42**(2): 94-100.

Dean H.T. and Elvove E., 1935. Studies on the minimal threshold of the dental sign of chronic endemic fluorosis (mottled enamel). Public Health Rep., **50**: 1719.

Gupta S.K., Gambhir S., Mithal A. and Das B.K., 1993. Skeletal scintigraphic findings in endemic skeletal fluorosis. Nucl-Med-Commun., **14**(5): 384-390.

Hui X., Yu-Lai Z., Zhang J.M., Liu H., Jing L. and Li G.S., 2007. Effects of fluoride on the intracellular free Ca2+ and Ca2+-ATPase of kidney. Biological Trace Element Research, **116**(3): 279-287.

Karen E., Haneke M.S. and Bonnie L., 2001. Sodium Hexafluorosilicate[CASRN 16893-85-9] and Fluorosilicic Acid (CASRN 16961-83-4). Review Toxicological Literature.

Kumari D.S. and Rao P.R., 1991. Red cell membrane alterations in human chronic fluoride toxicity. Biochemistry International, **23**(4):639-48.

Machalinska A., Wiszniewska B., Tarasiuk J. and Machalinskia B., 2002. Morpholgical effects of sodium fluoride on hematopoietic organs in mice. Fluoride, **35**(4): 231-238.

Mithal A., Trivedi N., Gupta S.K., Kumar S. and Gupta R.K., 1993. Radiological spectrum of endemic fluorosis: relationship with calcium intake. Skeletal-Radiol., **22**(4): 257-61.

Podder S., Chattopadhyay A., Bhattacharya S.and Ray M.R., 2010. Histopathology and cell cycle alteration in the spleen of mice from low and high doses of sodium fluoride. Fluoride, **43**(4): 237-245.

RGNDWM. Prevention & Control of fluorosis in India. Water Quality and Defluoridation Techniques, Volume II, Published by Rajiv Gandhi National Drinking Water Mission, Ministry of Rural Development, New Delhi, 1993.

Sun Z., Niu R., Wang B., Jiao Z., Wang J., Zhang J., Wang S. and Jundong W., 2011.Fluoride induced apoptosis and gene expression profiling in mice sperm in vivo. Archives of Toxicology, **85**(11): 1441-1452.

Takamorim T., 1962. The Heart Changes in Growing Albino Rats Fed on Varied Contents of Fluorine. The Toxicology of Fluorine Symposium, Bern, Switzerland: 125-129.

Tatin F., Grise. F, Reuzeau E., Genot E. and Moreau V., 2010. Sodium fluoride induces podosome formation in endothelial cells. Biol Cell, **102**(9): 489-98.

Vilber A.O., Bello and Hillel J. Gitelman., 1990. High Fluoride Exposure in Hemodialysis Patients. American Journal of Kidney Diseases, **15**: 320-324.

Wang Y., Yin Y., Gilula L.A. and Wilson A.J., 1994. Endemic fluorosis of the skeleton: radiographic features in 127 patients. AJR-Am-JRoentgenol, **162**(1): 93-8.

Wang A.G., Xia T. and Chu Q.L. et al., 2004. Effects of fluoride on lipid peroxidation, DNA damage and apoptosis in human embryo hepatocytes. Biomedical and Environmental Sciences, **17**(2): 217-222.

Wójciak-Stothard B., Potempa S., Eichholtz T. and Ridley A.J., 2001. Rho and Rac but not Cdc42 regulate endothelial cell permeability. J Cell Sci. **114**(7): 1343-1355.

Yoshisa Y., 1959. Experimental Studies on Chronic Fluorine Poisoning," Japanese Journal of Industrial Health. 1: 683-690.

Yu R., Xia T., Wang A. and Chen X., 2002. Effect of selenium and zinc on rat renal apoptosis and change of cell cycle induced by fluoride. Zhonghua Yu Fang Yi Xue Za Zhi. **36**(4): 219-21.