

# Histopathological studies on the spleen of guinea pigs during experimental fluorosis

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Abstract : Fluoride is a well-known environmental pollutant worldwide getting entry into the body mainly through drinking water. Excessive intake of fluoride results in all the three forms of fluorosis viz. dental fluorosis, skeletal fluorosis and non-skeletal fluorosis. Our present experiment is designed to study the histopathological effects of sodium fluoride on the spleen in adult guinea pigs given orally 50 mg NaF/kg body weight per day for 65 days. Abnormalities in the spleen showed damaged trabecular appearance and prominent cracks in both white pulps and red pulps. The lymphatic nodules and periarterial lymphatic sheaths were very much obliterated. The number of lymphocytes were increased and iron pigments decreased in the red pulps. The lymphatic nodules were devoid of germinal centers and the overall splenic tissue was highly basophilic. These alterations in the spleen due to fluoride intoxication may lead to reduced immunogenic response and decreased hematopoiesis as observed in fluorosis patients from endemic zones.

Key Words: HIstopathological, Spleen, Guinea pigs, Fluorosis.

#### **INTRODUCTION**

Fluoride is a well-known environmental pollutant. Fluorosis, a crippling disease due to the intake of excessive fluoride mainly through drinking water, is prevalent in several parts of the world including India. Fluoride poisoning results in three forms of fluorosis namely: Dental fluorosis, Skeletal fluorosis and Non-skeletal fluorosis which have been reported from many Indian states including Bihar. Scientists, toxicologists and physicians have devoted much attention to the problem of long term exposure of fluoride compounds in our body and their ill effects.<sup>1-5</sup> For the past few decades, significant number of research works have been done on the toxic effects of fluoride on organs 6-11 like liver, spleen, kidneys and bone marrow that actively participate in hematopoiesis. Roholm, in his animal experiments, encountered definite anemia in calves and dogs from chronic fluoride

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intoxication<sup>12</sup>. In a similar finding, Ginn and Volker<sup>13</sup> reported a nearly 30% reduction in blood haemoglobin of an unspecified strain of rats after 86 days with 50 ppm fluoride in their drinking water. In an other experiment on winstars rats for a period of three months, Belly<sup>14</sup> found that the lymphoid tissue mass of the spleen decreased and the relative proportion of reticulin and collagen structures increased. Similar findings were reported in mice treated with NaF as increased lymphocyte nodules, decreased white pulp and increased red pulp infiltration by lymphocytes<sup>6</sup>. Decrease in protein synthesis due to the genetic inactivation in rabbit fed on NaF has also been reported<sup>15</sup>. On a more physiological and biochemical side, fluoride has been found to effect erythrocytic membrane transport systems <sup>16</sup> and to enhance generation of superoxide radicals and lipid peroxidation in tissues of fluorosed animals leading to alteration in cell membrane structure and function <sup>17-19</sup>. Being the largest lymphoid organ and producer of about 20- 25 % of total blood cells <sup>20</sup>, Spleen plays a vital role in hematopoiesis <sup>21</sup> especially during foetal life in humans. Although the previous studies

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give ideas about the possible association between fluoride toxicity and haematological disorders, certain contrary findings as cited by Mc Clure and Kornberg<sup>22</sup>, Uslu <sup>23</sup> and Agate et al <sup>24</sup> along with similar other conflicting reports are causing problems in providing reliability and authenticity about them. Thus the present study aims to assess all the histopathological findings in spleen due to fluoride toxicity from the clinical point of view so that the potential influence of fluoride on the haematological diseases could be firmly established.

### **MATERIALS AND METHODS**

The experiment was performed on male and female guinea pigs weighing 243-420 gm each. All animals were acclimatized for at least one month before the day of dosing. The animals were randomly divided into two groups containing 6 guinea pigs each. The animals were housed individually in cages under constant climatic conditions. Feeding was ad libitum with a standard animal diet and drinking water was provided from normal low fluoride (<0.5 ppm) tap water also ad libitum. Guinea pigs of one group were treated with a solution of NaF (E. Merck (India) Ltd; Mumbai) in distilled water at a dose of 50 mg/kg bw/day orally and the second group served as control. The animals were anaesthetized with ether and sacrificed after 65 days of treatment along with age and sex matched control guinea pigs. The abdominal cavity was opened and spleen was dissected out. The organ was washed briefly in normal physiological saline and cut into pieces.

For histopathological studies the tissues were fixed in Carnoy's solution for 3 hours. After fixation, the tissue were dehydrated in graded series of alcohol, cleared in xylene, and embedded in paraffin wax (E. Merck (India) Ltd; Mumbai). Serial sections were cut at 5im and stained with standard hematoxylin and eosin. Microscopic slides were examined under a light microscope and subsequently photographed.

#### **RESULTS AND DISCUSSION**

The substance of the spleen consisting of both the white pulps and red pulps presented damaged trabecular appearance in the treated guinea pigs (Fig.1) in contrast to the control group (Fig.2). The spleen of control had normal pattern of venous sinuses, red pulps and white

pulps with normal germinal center. Iron pigments, the breakdown product of the old red blood cells, sequestered by macrophages were observed in large amounts. However, degeneration of splenic parenchyma which were highly basophilic and enlarged submarginal sinus around the white pulps with devoid of germinal centers were observed in fluoride treated animals. The capsule covering the spleen was seen intact and normal in the control guinea pigs (Fig.3) in comparison to the treated animals where it was extensively damaged (Fig.4). Contents of white pulps, particularly lymphocytes in lymphatic nodules and periarterial lymphatic sheaths were decreased in treated animals. Structure of many lymphatic nodules and periarterial lymphatic sheaths were very much obliterated and presented a cracked appearance (Fig. 5). The number of lymphocytes in the red pulps were increased in treated groups as compared to the control. Macrophage activities and iron pigments in the red pulp area were less pronounced in the treated guinea pigs in comparison to the control animals. Intermingling of red pulp and white pulp with indistinct marginal zone was also noted.

The histopathological changes in the spleen due to sodium fluoride during the present studies may have marked negative effects on the immune system of the body. Inhibition of the immune system with fluoride by confusing it and causing it to attack the body's own tissues has been reported<sup>25-27</sup>. Inhibition of antibody formation in the blood of rabbit treated with fluoride has been shown<sup>28</sup> which may be due to the obliteration of lymphatic nodules and germinal centers as observed during present investigation. Fluoride has been reported to decrease the ability of white blood cells to properly destroy foreign agents by process of phagocytosis<sup>29-30</sup>. During the present study, the activities of the macrophages were less pronounced as evident by decreased amount of iron pigments in the red pulps area of the spleen of fluoride treated guinea pigs. In the fluoride treated mice, morphological changes were seen in the spleen as increased lymphocyte nodules, decreased white pulp, and increased red pulp infiltrated by lymphocytes<sup>6</sup>.

Contents of the white pulps including lymphatic nodules and periarterial lymphatic sheath were seen reduced during the present observation in the guinea pigs treated with fluoride. In mice, this organ is actively

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involved in blood cell production throughout the life. The spleen is active as a site of hematopoiesis mostly in fetal life in case of human beings<sup>20-21</sup>. As erythropoietin is one of the strongest growth factors regulating red blood cell production and maturation in bone marrow<sup>31</sup>, the histopathological changes observed in the spleen during the present investigation may also have adverse effect on hematopoiesis. In rabbits treated with 10 and 50 mg NaF/ kg body weight for 2 to 4 weeks, markedly decrease total nucleated cell counts and anemia have been reported<sup>32</sup>. In industrial region polluted by fluoride, children had decreased hemoglobin levels<sup>33</sup>.Kaj Roholm in his classical



Fig 1 Photomicrograph of the section of spleen of treated guinea pigs showing damaged trabecular appearance of both white pulps and red pulps. The splenic cells are highly basophilic and present a degenerated appearance. Hematoxylin and Eosin Stain x100



Fig 3 Photomicrograph of the section of spleen of control guinea pigs showing normal venous sinuses and splenic cords with large amount of deep staining iron pigments. The normal splenic structures are covered by intact capsule of dense connective tissue. Hematoxylin and Eosin Stain x100.

studies found a small reduction in the erythrocyte count and slight anemia among cryolyte workers, but observed definite anemia in calves and dogs from chronic fluoride intoxication during animal experiments<sup>12</sup> These observations are in conformity with our present studies.

It is concluded that the histopathological changes observed in the spleen due to fluoride toxicity during present investigation may cause reduced immunologic competency and decreased hematopoiesis as observed in fluorosis patients. These findings may have direct bearing on the people living in the endemic areas for fluorosis.



Fig 2 Photomicrograph of the section of spleen of control guinea pigs showing normal histological structure with well organized periarterial lymphatic sheath and lymphatic nodule of white pulp with central artery. Venous sinuses and splenic cords of the red pulp are clearly seen as normal structure. Hematoxylin and Eosin Stain x100.



Fig 4 Photomicrograph of the section of spleen of fluoride treated guinea pigs showing extensive damages to the capsule of dense connective tissue covering the spleen. The submarginal sinus around white pulp is enlarged and lymphatic nodules are devoid of germinal centres. Note that the number of lymphocytes are increased and iron pigments decreased in the red pulps.

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Fig 5 Photomicrograph of the section of spleen of fluoride treated guinea pigs showing extensive damages to the splenic parenchyma presenting prominent cracks and trabecular appearance indicating large scale degeneration of the splenic tissues. Lymphatic nodules are devoid of germinal centres.

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