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Sodium fluoride induced changes in body weight and liver histology of albino: Rabbit

[*1] Santosh K Sahu, [2] Sujita Pradhan
[1] Assistant Professor, Department of Anatomy, SCB Medical College, Cuttack, Odisha, India
[2] Tutor, Department of Anatomy, IMS and SUM Hospital, Siksha 'O' Anusandhan University, K8, Kalinga Nagar, Bhubaneswar, Odisha, India

Abstract
Fluoride is an environmental pollutant present in dissolved state largely in many sources of drinking water affecting many organs in human being when ingested in high levels for prolonged period. Effects of sodium fluoride in liver cytoarchitecture of albino-rabbit was studied in the present experimental work as well as its effect on gross body structure. Six adult healthy albino-rabbits were divided into 3 groups (2 rabbits per each group). The first group served as control group and received de-ionized water. The second group was treated with 0. 5% of sodium fluoride solution and third group was treated with 3% of sodium fluoride solution for 4 months. At the end of the experiment, the animals were sacrificed by ether anaesthesia and livers were dissected out and processed for histopathological study. The present experimental work showed that exposure to sodium fluoride for 4 months in high doses resulted in degenerative changes in liver cytoarchitecture and also loss of body weight and skin rashes all over the body.

Keywords: albino-rabbit, loss of weight, Histopathalogical changes, sodium fluoride, liver

1. Introduction
Trace elements are essential and beneficial to human health in minute concentrations, as they play an important role in many metabolic processes and act as co-factors. However, exceeding their permissible intake, is known to be toxic and has adverse effect on the general body metabolism. Fluorine, is one such trace element, which is ubiquitously distributed in the environment [1]. Combined chemically in the form of fluorides, it is seventeenth (17^{th}) in the order of abundance of elements, in the earth's crust. Sea water itself, contains significant amounts of fluoride levels, having been variously recorded as 0.8-1.4 mg/l (Wattenberg, 1943; Kappan *et al.* 1962). Additional fluorides are widely distributed in the atmosphere, originating from the dust of fluoride-containing soils, gaseous industrial wastes, burning of coal in populated areas, and from the gases emitted in areas of volcanic activity. Fluoride problem occur with releasing of fluoride dust and fumes from different industries using hydrofluoric acid and fluoride salts [2]. There are many kinds of fluoride ingestion by man such as fluorides in solid food (Vegetables and fruits, cereals, barley, rice, meat, fish), fluorides in drinks (drinking water, human breast milk, tea leaves, fresh fruit juices) and fluoride inhaled from the air. However, the effects of fluoride on the health of man, stem largely from dissolved fluoride present in many sources of drinking water. With oral route along with food and water, fluoride is found in small quantities in almost all foods and enters into the human body [3]. Consumption of fluoride over a long period of time affects the soft tissues like liver, kidney, brain, muscle tissue, gastrointestinal tract and several other reproductive and endocrine organs by the property of simple diffusion and leads to impairment of soft tissues [4, 7]. People of several countries including India have suffered from severe fluorosis due to ingestion of sodium fluoride leading to a major public health hazard [8]. Recent study has demonstrated that decreased aerobic metabolism causes accumulation of fluoride and inhibit the Kreb's cycle leading to liver toxicity [9, 12]. Earlier studies have found that fluoride causes degenerative and inflammatory changes, dilatations of sinusoids, hepatic hyperplasia and accumulation of amorphous and crystalline bodies in the hepatocytes in liver [13, 14]. This study is also worked out to examine the fluoride-induced hepatic damage in albino- rabbits.

2. Materials and Methods
The present study had been undertaken in the Department of Anatomy, S.C.B. Medical College, Cuttack, in collaboration with Department of Pharmacology and Department of Pathology, S.C.B. Medical College, Cuttack and IMS and SUM Hospital, Siksha 'O' Anusandhan University, K8, Kalinga Nagar, Bhubaneswar. Ethical clearance was obtained from the Institutional Animal Ethical Committee.
Healthy adult male albino rabbits weighing 1.5 – 2 Kg. were kept at laboratory conditions in well cleaned cages. The animals received standard rabbit-feed and ad-libitum tap water. Rabbits were divided into three groups A, B and C containing two animals in each group. Group A animals served as controls and received de-ionized water. Group B animals were treated with 0.5% sodium fluoride solution (5 mg/kg body wt.) through oral route daily up to four months. Group C animals were treated with 3% sodium fluoride solution (30 mg/kg body wt.) through feeding tube orally for four months. After the end of the experiment (i.e after 4 months), the body weights and other gross structural changes

were recorded and sacrificed by ether anaesthesia. Livers were dissected out immediately and kept in a fixative of neutral formalin. Liver tissues were processed for histopathological study and paraffin sections of 3-5 µ thick were prepared with the help of microtome and prepared slides were stained with Harris - Haematoxylene and Eosine. Glass slides containing the liver tissue sections were mounted with DPX and observed under low power light microscope.

3. Results

Gross structural changes were absent in both Group-A and Group-B rabbits after the experiment. However, after 4 months of exposure to Sodium Fluoride to Group-C rabbits showed marked reduction of gross weight, lethargic and inactive attitude with skin rashes all over the body (Fig.5 & Fig.6).

Histological changes in the livers of both Group-B and Group-C rabbits,following continuous daily exposure to sodium fluoride solutions in two different doses (i.e. 0.5% solution for Group-B and 3% solution for Group-C rabbits) for 4 months were studied in detail and compared with those of the control (Group-A) rabbits.

Total six (6) rabbits of all the groups (i.e. Groups-A, B & C) were sacrificed at the end of the experiment (i.e. 4 months). In Group-B rabbits the histological findings remained unchanged after the experiment as was observed in case of control Group-A. However, in Group-C rabbits the histological findings changed, which have been tabulated below in Observation table.

The histology of liver in Group-A and Group-B rabbits show normal hepatic cords of cells, with more or less centrally placed nuclei and homogenous cytoplasm.Vascular sinusoids running in between the parenchyma cells are observed in the liver cytoarchitecture. Normal portal triads are seen in periportal area (Fig.3 & Fig.4).

The histological findings of liver in Group C rabbits exposed to sodium fluoride for 4 months in high doses has shown remarkable changes as compared to controls and Group-B rabbits, such as cellular distortion of hepatic cords, severe necrosis in hepatocytes, nuclear fragmentation, pushing of nucleus to periphery of hepatocytes, hemorrhage in central vein, congestion & dilatation of portal sinusoids,ballooning degeneration of hepatic cells, centrilobular necrosis, and round cell infiltrations (Fig.7 to Fig.10).

4. Discussion

Liver is the vital organ for metabolism and involved in the metabolism of toxic compounds produced during systemic biochemical processes and exogenous toxins entering into the organisms from the environment [15]. Sodium fluoride would induce both pathomorphological and metabolic changes in liver [16]. Liver was severely damaged by sodium fluoride in the present study on albino-rabbits and revealed, cellular disarray, congestion, ballooning degeneration and cellular vacuoles, nuclear degeneration, honeycombed liver with round cell infiltration. Several studies show similarity with our results [17, 18, 19, 20]. Shashi and Thapar have found hepatocellular necrosis, hepatic hyperplasia, extensive vacuolization in hepatocytes, dilation of central vein and sinusoids in liver of albino rabbits exposed to sodium fluoride as is found in our study [21]. Smyth and Smyth repoted degeneration of hepatic cells in rats intoxicated with barium fluorosilicate [22]. Degeneration of hepatic cells especially around hepatic vein and accumulation of both amorphous and crystalline bodies in the liver of experimental animals were observed by Phillips *et al.* [23]. Chinoy *et al.* showed zonal necrosis and pycnosis of hepatocyte nuclei and disturbed arrangement of hepatic cords in fluoride-treated rats [24]. Bogdanffy *et al.* reported hepatic hemangiosarcoma, hepatocellular adenoma and carcinoma, hepatic foci of clear cell and asophilic alteration in rats and mice exposed up to 2500 ppm vinyl fluoride [25]. Fluoride induces hepatotoxicity in rabbits as evidenced by oxidative stress [26]. In addition, Trivedi also reported that cellular necrosis and degeneration in liver is due to significant increase in serum glutamate oxalate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) levels in rabbit after oral administration of sodium fluoride for 30 days [27]. It is believed that increased levels of SGOT and SGPT lead to liver damage [28]. Many observations are similar to our results [29, 30]. In fluorotic patients, hepatic cellular necrosis with round cell infiltration has been recorded which were also found in the present study [31]. Honey-combed liver where hepatic cells were separated from each other with marginal cytoplasm and centrilobular necrosis were found in our study. Haber even found mid-zonal hepatic cell necrosis caused by fluoride ions in patients receiving fluorinated anesthetics [32].

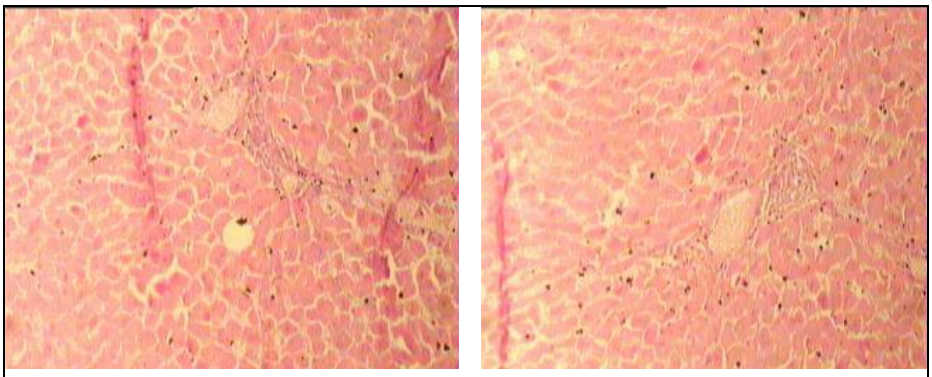
Table 1: Gross changes and Histopathological changes of liver of all the 3 groups after treatment of Sodium fluoride solution.

S. No.	Experimental Groups	Duration of Naf exposure	Gross and histological changes Observed
1	Group-A	4 Months	No Changes
2	Group-B	4 Months	No Changes
3	Group-C	4 Months	Gross Changes - Loss of weight, lethargic and inactive attitude with skin rashes. Histological Changes - Distortion of hepatic cords of cells, necrosis & ballooning degeneration of hepatic cells, congestion & dilatation of portal sinusoids, honeycombed liver with focal infiltrations of inflammatory cells around dilated sinusoids.

Note: NaF= Sodium fluoride.

Fig. 1 Rabbit weighing > 1.5Kg. Fig.2 Healthy active rabbit

Fig 1 & 2: Before the experiment



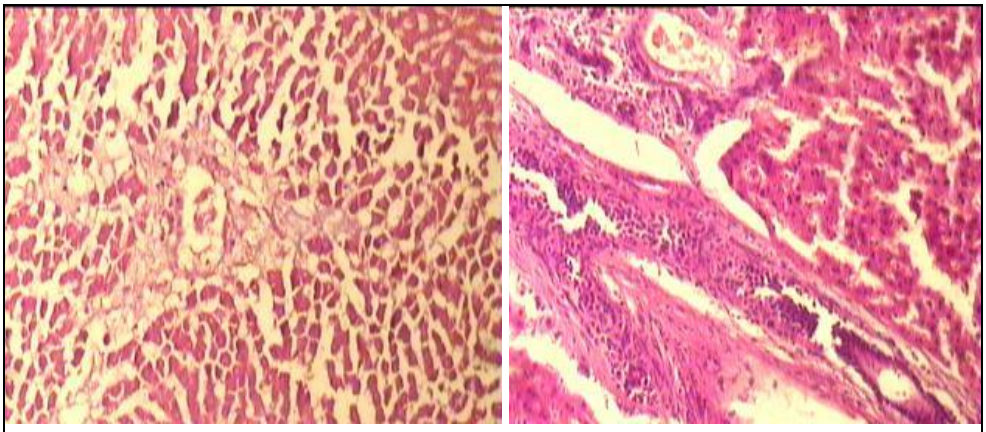
10 X Magnification

Fig.3 Control rabbit before the experiment Fig.4 Group-B rabbit after the experiment

Fig 3 & 4: Photomicrographs Showing normal liver cytoarchitecture

Fig.5 Reduction of weight of Gr. C Rabbits Fig.6 Lethargic Gr. C rabbit showing skin rashes

Fig 5 & 6: After the experiment



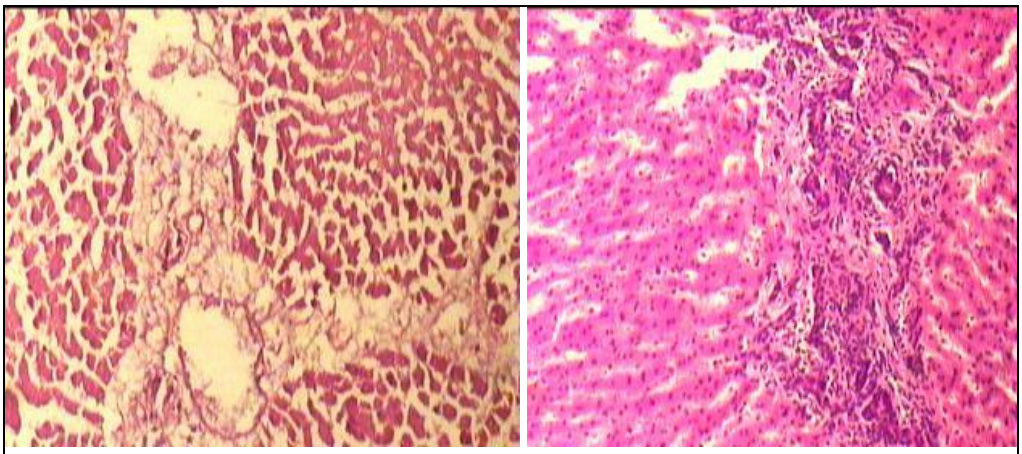
10 X Magnification

Fig.7 Liver Photomicrograph of Gr.C Rabbits showing distortion and degeneration of hepatic cellular cords with centrilobular necrosis.

10 X Magnification

Fig. 8 Liver Photomicrograph of Gr.C Rabbits showing ballooning degeneration of hepatic cells with congested and dilated sinusoids.

Fig 7 & 8: After the Experiment



10 X Magnification

Fig.9 Liver Photomicrograph of Gr.C Rabbits showing honeycombed hepatic cells with marginal cytoplasm.

10 X Magnification

Fig.10 Liver Photomicrograph of Gr.C Rabbits showing dilated sinusoids with round cell infiltrations.

Fig 9 & 10: After the Experiment

5. Conclusion

From the results, it was concluded that sodium fluoride exposure to rabbits in high doses for prolonged period affects gross body weight and has direct effect on liver parenchyma exhibiting severe hepatotoxicity as compared to rabbits exposed to sodium fluoride in low doses exhibiting no changes in liver Cytoarchitecture and gross body weight.

Conflict of Interest - None declared.

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