Toxicity And Risk Assessment of Sodium Fluoride at LD_{50} Concentration

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Abstract
The study examined the toxicological effects of acute toxic Sodium fluoride (NaF) doses on Swiss albino mice. The lethal dose (LD_{50}, which is the amount of a substance that is lethal to half of a group of test animals) method was used to determine the acute poisonous dose of NaF. The acute toxicity of NaF was tested on 4 healthy mice weighing 20-25 grams. The test was performed thrice to confirm the results—an arbitrary dose of 20mg/kg b.w. NaF dissolved in 75 ml of distilled water was administered orally to the mice. The mice died after 24 hours of oral NaF administration. In Swiss albino mice, the approximate fatal dose of Sodium fluoride was 20 mg/kg b.w. A post-mortem investigation was performed on 50% of the mice that died during the experiment, and the mice were monitored for clinical indications of toxicity. As a result, the mice exhibited prostration, piloerection, sparse droppings, gasping, cyanotic paws, stooped posture, and loss of reflexes. Microscopic examination revealed histopathological changes in the liver, kidneys, and brain., It showed severe degenerative and necrotic alterations in neurons of the brain, degeneration and necrosis of the kidney's tubular epithelium and glomeruli, and dilated sinusoids in the liver.

Keywords: Histopathology, LD_{50}, Necrotic alterations, Necrosis, Post-mortem, Swiss albino mice, Sodium fluoride, Toxicological effects.

Introduction

Fluorine, the 17th most abundant element in the Earth's crust, is highly reactive and primarily found in minerals such as aluminum, iron, calcium, silicates, and phosphates. As a result, fluoride is a key component of many rocks and ores, constituting approximately 0.06–0.09% of the Earth's crust (Guth *et.al*.,2020). Fluoride-rich groundwater forms through the weathering of primary rocks and the leaching of fluoride-containing minerals in soils (Wasa *et.al*., 2024).

Fluoride exposure occurs mainly through fluoridated water, dental products (toothpaste, fluoride tablets, and drops), and groundwater contaminated by geological sources (Federica *et.al*.,2023). Additionally, all vegetation absorbs fluoride at low concentrations, with higher levels detected in tea, kale, fish, barley, and rice. However, excessive fluoride intake can lead to endemic fluorosis in areas with high fluoride concentrations in water. Industrial fluorosis arises from inhaling fluoride polluted air near industries such as aluminum and steel manufacturing, fertilizer production, brick kilns, and ceramic factories.

Once inside the body, fluoride crosses cell membranes and affects various tissues, including the heart, liver, skin, and erythrocytes, by increasing oxidative stress (Agalakova *et.al*., 2020) It inhibits key enzymes in cellular energy metabolism, reducing ATP levels and impairing carbohydrate, protein, nucleic acid, and lipid metabolism. Moreover, fluoride contamination in the environment is a potential biohazard for plants and animals due to its widespread use in fluoridated water (Saha *et.al*., 2024). Fluoride disrupts DNA and protein synthesis at high doses, inhibiting cell proliferation and causing cytotoxic effect (Santesso *et.al*.,2021). Acute fluoride toxicosis, particularly through ingestion of sodium fluoride (NaF), can lead to severe pathological effects on visceral organs (Raghuvanshi *et.al*.,2010)

This report investigates the approximate lethal dose of NaF in mice, detailing observed clinical signs and histopathological alterations in visceral organs associated with acute NaF toxicity.

Materials and Methods:
The acute toxicity of NaF was tested on 4 healthy Swiss albino mice weighing 20-25 grams and the test was performed thrice to confirm the results. The lethal dose (LD_{50}) method was used, and an arbitrary dose of 20mg/kg b.w. NaF dissolved in 75 ml of distilled water was administered orally to the mice. Approval was obtained from the Institutional Animal Ethics Committee (IAEC) before conducting the study. The mice were observed for any clinical signs of toxicity, and 50% of those who died during the study were subjected to post-mortem examination. The remaining 50% of mice that survived were also monitored. Tissue samples were collected for histopathological studies, and paraffin-embedded tissue sections of 4-5µm were obtained and stained with hematoxylin and eosin for analysis.

Observations and Results:
The approximate lethal dose of Sodium fluoride was determined to be 20mg/kg b.w., per single administration in Swiss albino mice. The first group of mice intoxicated with an initial arbitrary dose of 5 mg/kg b.w., orally showed mild irritation and hyperactivity just after administration of the dose, and the second group exposed to 10 mg/kg b.w. exhibited the signs of mouth smacking, mild irritation, and hyperactivity. However, the mice recovered to normalcy after a short time gap of 12 minutes. The third group intoxicated with 15 mg /kg b.w. showed irritation, and hyperactivity (2 hours post-exposure). However, the 3 mice recovered after 8 hours from all these signs and assumed normal functions, and 1 died after 24 hours of exposure. Therefore, the fourth group was intoxicated orally with a higher dose of 20 mg/kg b.w., The toxic signs appeared instantaneously following its administration and became more pronounced with time. The toxic signs noticed after 3 hours and 20 minutes comprised prostration, piloerection, and the foot pad became cyanotic (fig. 1). 50% of mice died after 24 hours of oral NaF intoxication. The mice died following intoxication with 20mg/kg b.w., as the noted dose was considered an approximate lethal dose for Swiss albino mice.
Therefore, histopathological examination was conducted on animals with a 20mg/kg b.w dose.

Fig.1. Cyanotic foot pad (lack of oxygen in the blood) of mice exposed to an approximate lethal dose i.e., (20mg/kg b.w) of NaF

- Histological sections of the liver of the treated group (LD_{50})
(Hematoxylin and eosin-stained slides at 40x magnification)

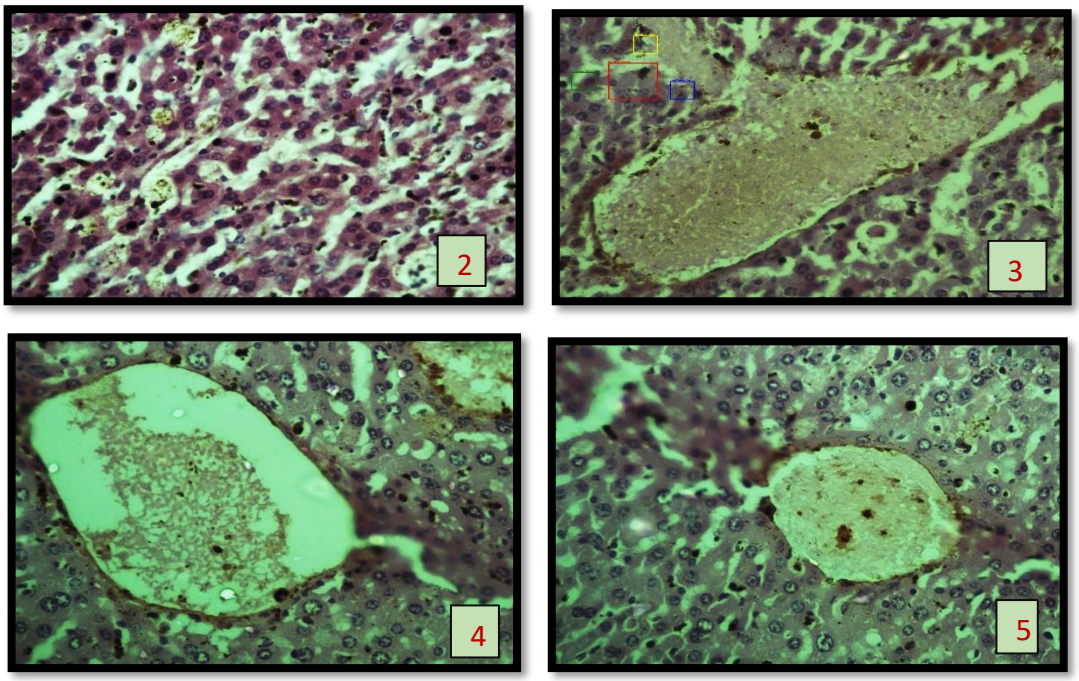

Fig. 2. T.S. section of liver showing cellular changes, Sinusoidal Congestion, Fig. 3. inflammatory cell, disorganization of hepatocytes, Fig. 4. Section of the liver slide showing deposition of brownish pigment, Fig. 5. the central pale area representing bile stasis.

- Histological sections of the kidney of the treated group (LD_{50})
  (Hematoxylin and eosin-stained slides at 40x magnification)

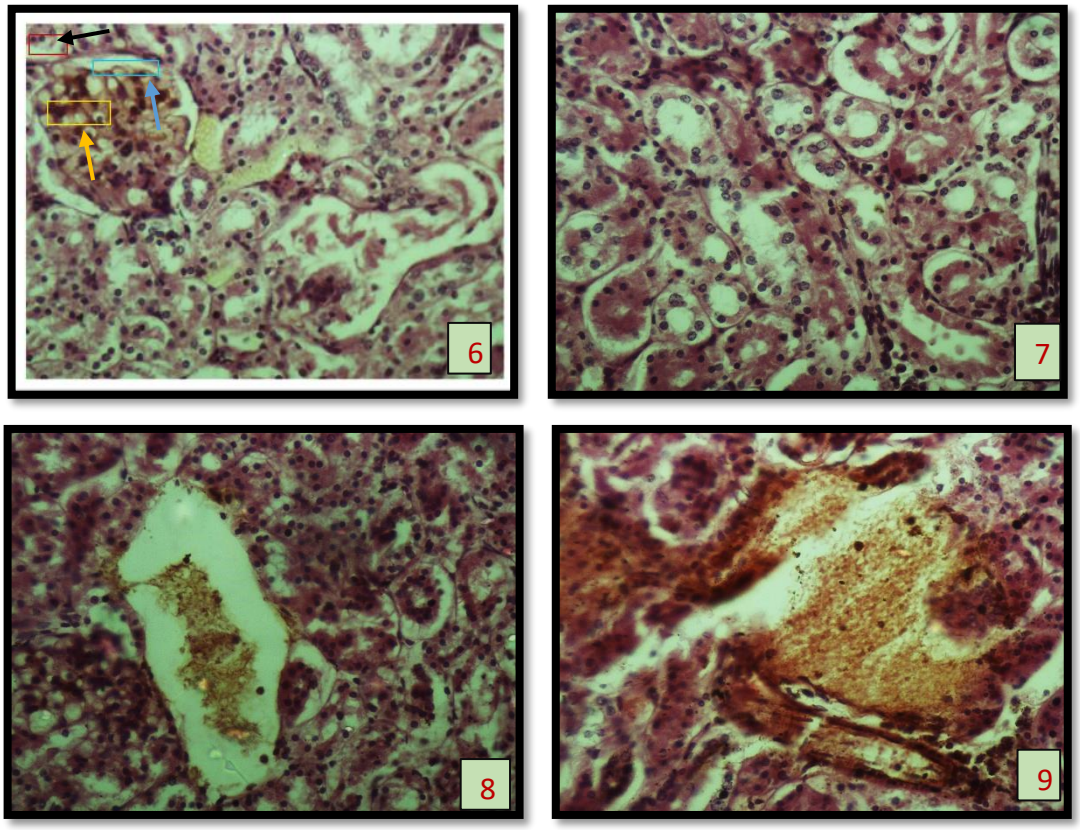

Fig. 6 T.S Section of Kidney showing glomerular, tubular, interstitial changes, Fig. 7. Cytoplasmic vacuolization, tubular dilations, Fig. 8. Tubular structures appear disorganized, Fig. 9 Cellular disarray.

- Histological sections of the brain of the treated group (LD_{50})
  (Hematoxylin and eosin-stained slides at 40x magnification)

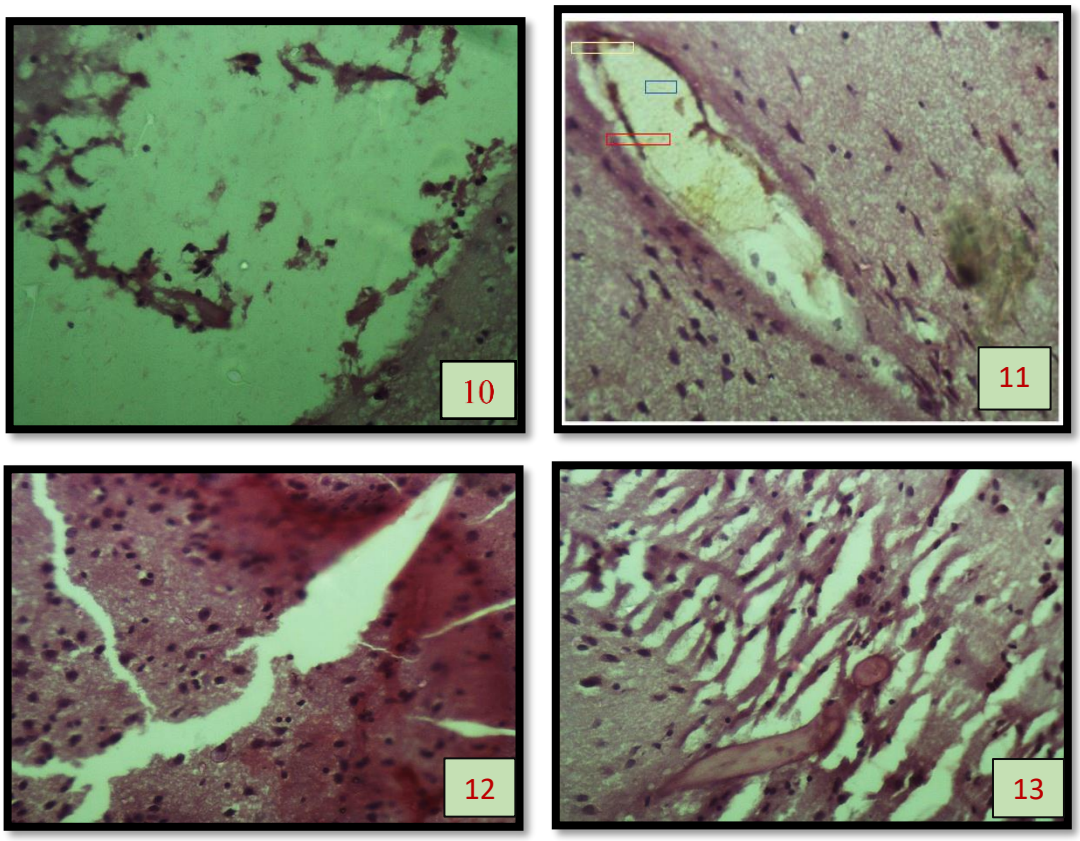

Fig. 10. Brain section showing Neuronal damage, Fig. 11. Sodium fluoride toxicity leads to damage of neurons, Fig. 12. The white areas represent vacuolization, and Dark condensed nuclei suggest apoptosis, Fig. 13. Separation of neuronal layers.

The necropsy findings of acute toxicity were generalized congestion in the liver, kidney, and brain. Histopathological screening of the liver sections shows cellular changes (dark-staining nuclei indicating cell stress, cells are irregular in shape, Cytoplasmic changes are visible, with some cells showing granular or vacuolated cytoplasm, which signifies hepatocyte degeneration or toxic injury, and Sinusoidal Congestion (indicate vascular damage or impaired blood flow due to hepatocyte swelling) and Necrosis (cell death caused by harmful effects of NaF) (Fig.2). Sodium fluoride (NaF) showing toxic effects on the liver, Necrotic tissue regions (degenerative), inflammatory cell infiltration, disorganization of hepatocytes, fatty changes (Fig.3). In another T.S section of liver, it is showing the light-colored central structure showing coagulative necrosis (hepatocytes loss) and fibrotic regions (chronicity of injury), brownish pigment deposit showing hemosiderin (iron accumulation), and bile pigment (due to cholestasis), ballooning degeneration (early apoptosis) (Fig.4). The another T.S. slice demonstrates the harmful effects of NaF on the liver, which include inflammation, cell destruction (damaged hepatocytes), bile stasis (shown by the middle pale area), and possible lipid buildup (Fig.5). Histopathology of kidneys revealed glomerular, tubular, and interstitial changes (fibrosis, deposition of extracellular matrix), inflammatory response (indicating a harmful response), and Vascular damage (blood vessel damage) (Fig.6). T.S section of Kidney showing Cytoplasmic vacuolization (toxic nephropathy), tubular dilations (stress caused by toxins), interstitial and inflammatory signs: Edema (swelling, suggesting toxic

damage affecting fluid dynamics), cell shedding (detachment from the tubular basement membrane) (Fig.7). In another slide of Kidney showing the presence of an amorphous deposit within the lumen of a vessel suggests potential proteinaceous material, debris, or clot formation, tubular structures appear disorganized, possibly indicative of necrosis or apoptosis, suggesting degenerative effects (Fig. 8). In another T.S section of Kidney showing, the intense eosinophilic staining suggesting protein aggregation (cellular damage), cellular disarray (areas seem to lack defined tubular structures), inflammatory signs indicating tissue response to injury (Fig.9). The histopathological sections of the brain showed Neuronal damage (pyknosis, and loss of neuronal density), Vascular disruption, Gliosis, and Oxidative stress (Fig.10). There was damage to neurodegeneration(hemorrhage), Potential necrosis (oxidative stress leading to neuronal cell death), and neuronal cells (toxicity causes shrinkage, and vacuolization) (Fig.11). The clusters appeared as cell nuclei, potentially indicating areas of stress or damage, the white areas representing vacuolization, and Dark, condensed nuclei suggesting apoptosis (Fig.12). Another brain slide displaying the presence of active microglia, neuronal layer disarray, or separation (Fig.13).

Discussion:

The World Health Organization (WHO) reported that the oral LD_{50} of NaF in rats was between 68.584 and 223.45 mg/kg b.w. during 24 hours (WHO,2002). The hepatocellular toxicity caused by fluoride-induced inhibition of many enzymes involved in ATP generation and lipid peroxidation may be the cause of the histological abnormalities in the liver (Dutta *et.al*.,2007). The activation of platelets, including their adherence, shape change, aggregation, and secretion, may be the cause of the thrombus formation in sinusoids in the liver (Gassowska *et.al*.,2010). The kidneys are the primary organs responsible for removing fluoride from the body (Giri *et.al*.,2014). These are particularly susceptible to the harmful effects of fluoride because they have a higher concentration of fluoride in the filtrate. Overexposure to fluoride damages kidney structure and function, mainly affecting the proximal convoluted tubule epithelial cells (Dote *et al*.,2000). The suppression of cellular enzymes and fluoride induced hypocalcemia, potential necrosis, disorganization of neurons, and neurodegeneration could be the causes of the acute effects of fluoride on the central nervous system (Augenstein *et al*.,1991).

Conclusion:

The results of this study provided guidelines for selecting doses for subacute and chronic toxicity studies of NaF in laboratory mice. Based on the findings of this study, it is concluded that the approximate lethal dose of NaF in Swiss albino mice is 20 mg/kg b.w., orally, as a result, the mice exhibited prostration, piloerection, sparse droppings, gasping, cyanotic paws, stooped posture, and loss of reflexes and also Various histopathological alterations in visceral organs indicate nephrotoxic, hepatotoxic, and hemorrhage (neurodegeneration).

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