Ameliorative Effects of Orange Juice on Sodium Fluoride Induced Gastro-Hepatic Toxicity in Mice

Ata ul Mustafa Fahid¹, Azeem Azam¹, Farhan Anjum¹, Hamza Faseeh¹, Rabia Bano², Sana Kausar¹, Attiq ur Rehman¹ and Maryam Latif¹

¹Institute of Zoology, Punjab University, Lahore, Pakistan
²Department of Zoology, Division of Science, University of Education, Pakistan

INTRODUCTION
Fluorosis is a worldwide endemic disease that affects multiple continents. Fluoride-related health problems in humans are becoming increasingly common due to natural and geological causes as well as more recently, rising industrialization and the resulting environmental contamination. Worldwide, drinking water containing high amounts of fluoride has been linked to health risks; in India alone, there are an estimated 66.62 million victims. Water that is consumed by humans and animals, along with food to a lesser amount, allows fluoride to enter their bodies. Fluoride exposure produces the anion superoxide (O2•-), which raises O2•- concentrations and has downstream effects on molecules including H2O2, ONOO• (an isomer of nitrate), and O-H reactive species. These molecules play a key role in mitigating the toxicity of fluoride ions. Vital organs such as kidney, liver, brain etc, are adversely affected, which has been demonstrated by laboratory testing. Fluoride lowers glutathione levels and prevents the actions of antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase. Oxygen radicals may be produced excessively at the mitochondrial level as a result of glutathione deficiency, which damages various parts of cells. Furthermore, the synthesis of too many oxygen radicals may result in peroxidation of lipids, oxidation of macromolecules, depolarization of the mitochondrial membrane and death.
[2]. Higher fluoride intake has been shown to cause metabolic disorders by interfering with a number of cellular processes such as transcription, translation, mitosis, many cellular chemical reactions and other basic cytological processes. Moreover, it compromises antioxidant defence system of the body [3]. Low amounts of the vital trace element fluoride (F-) have been shown to support the growth of teeth and bones [4]. It exists in the environment in a variety of forms and is widely utilized, as are its compounds. Water use generally accounts for the greatest portion of daily F- intake, either because F- containing rocks and soils runoff into water sources. Some regions use artificial fluoridation of drinking water or groundwater [5]. Thus antioxidant/pro-oxidant multi-organ dysfunctions may result from an imbalance brought on by fluoride intoxication [6]. The amount of fluoride taken up by plants is directly related to the kind of plant involved and also upon the strength of the polarity of nutrient medium upon which it is grown. After a certain threshold level of fluoride ions, the influx of this ion through roots may increase dramatically [7]. In an experiment it was observed that the concentration of fluoride was greater in roots as compared to that in shoots in tomatoes (Lycopersicumesculentum) and oats (Avena sativa) when experimentally grow on substrate media rich in fluoride [8]. Coal mines where fluoride concentrations are dangerously high can produce airborne fluoride particles in the concentrations of 6 µg/m3, which has been observed in parts of China [9]. The innate ability of sodium fluoride to induce procreative and embryological defects has also been investigated in laboratory animals [10, 11]. The blood chemistry and hepatic functions of many test animals exposed to higher levels of fluoride have been shown to cause fluctuations in the normal working of these parts [12]. A short-lived duration inhalation MRL, premised on respiratory tract agitation in humans was derived for hydrogen fluoride [13].

METH O D S

Sodium fluoride and natural fresh orange juice were used in this experiment for 30 days each.

The chemical composition of fluoride as shown in table 1.

<table>
<thead>
<tr>
<th>Table 1: Chemical composition of fluoride</th>
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<tr>
<td>Company Name's</td>
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<tr>
<td>Colour</td>
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<tr>
<td>Form</td>
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<tr>
<td>Highest Percentages of Impurities</td>
</tr>
<tr>
<td>Chloride (Cl)</td>
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<tr>
<td>Copper (Cu)</td>
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<td>Iron (Fe)</td>
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A group of 15 albino mice (10 females and 5 males) were obtained from Veterinary Research Institute, Lahore, at the beginning of experiment. Colony of albino mice was established to fulfill demands of experiment. These animals were kept in air-conditioned animal house at 25±1°C temperature. Steel racks and cages (14” × 10” × 7”) were also available for this purpose. Two females were caged with one male in five different cages. Breeding stock was kept in controlled environmental conditions in the form of 12-hour light/ dark cycles, temperature of 25±1°C and humidity i.e., relative 40-50%. Each cage had wood shavings as bedding material, which was replaced daily. Before the drug administration, animals were weighted and divided. Every group consisted of five animals. The animals were fed drinking water with sodium fluoride indifferent concentrations and drinking water with orange juice (in ml) daily as detailed above. Every animal was weighed weekly.

Control group i.e. G-I was supplied with Nestle’s water while treated groups (from G-II to G-VI) were first treated with various concentrations of sodium fluoride such as G-II 5-µg/g B.W (Body Weight), G-III with 7.5µg/g, G-IV with 2.5 µg/g, G-V with 2.5 µg/g sodium fluoride for 30 days and after that all treated groups were given fresh orange juice (50% in distilled water) for next 30 days. On the 31st day, animals were weighed and then anaesthetized with ether. The organs were dissected out of the animal, then fixed in Bouin’s fluid for 48 hours with subsequent preservation in 70% alcohol. Both control and treated organs were processed for morphological and anatomical studies. The organs were weighed by using digital balance, organ length and diameter were measured by using avernir caliper. The whole data were subjected to mathematical calculations and were analyzed by a computer-based program SPSS. One way ANOVA was used for analysis of data obtained in this study. The study was ethnically accredited by Examination branch, University of Education Township Lahore (REF#: 1090, Dated: 15-09-2013)

RESULTS

Morphological Studies

In Group-I, no abnormalities were observed in liver and stomach. All the livers and stomachs of albino male mice were normal and proper in morphological structure. Group-II revealed some abnormalities in liver such larger size of posterior right lobe, median lobe, gallbladder, caudate lobe and left lobe and stomach fundic region were narrower than control. In Group-III, posterior right lobe became small, gall bladder expanded and stomach effected as esophagus, cardia, fore-stomach, body, limiting ridge, region of fundic glands and region of pyloric gland showed abnormal structure. In Group-IV an infection of liver in posterior right lobe, median lobe, gallbladder, caudate lobe and left lobe and stomach regions showed abnormal appearances. In Group-V, cancer production occurred in cardia, fore stomach and
region of pyloric glands were affected and in Group-VI, Liver posterior right lobe, gallbladder, caudate lobe, right median lobe and left lob expanded in form and spots and hole formation were observed, infection occurred in forestomach, fundus, limiting ridge, region of fundic glands and region of pyloric glands of stomach.

Figure 1: Macrophotograph of liver of albino male mice from G-I to G-VI, showing no abnormalities in control (G-I) while treated Groups showing abnormalities Posterior right lobe (PR), anterior right lobe (AR), median lobe (M), gallbladder (G), caudate lobe (C), right median lobe (RM) and left lob (L).

Figure 2: Macrophotograph of stomach of albino male mice. G-I (control) showing no abnormalities while treated Groups showing abnormalities. Esophagus (E), cardia (C), fore stomach (FS), fundus(F), limiting ridge (LR), body(B), antrum(A), region of fundic glands (RF) and region of pyloric glands (RP)

Morphometric Analysis
The organs from all the groups; control and experimental, were subjected to morphometric analysis. The morphometric study of the organs was based on ONE-WAY ANOVA Duncan test by using SPSS-16 software. Analysis of different dose groups was made on the basis of different parameters. These parameters included wet weight, length, diameter and organ-somatic index.

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stomach diameter of Group–IV (2.50µg/g B.W NaF) was 5.91±0.50 mm which was decreased significantly (p<0.05) than control. The average stomach diameter of Group–V (5.00µg/g B.W NaF) was 5.63±0.45 mm which was decreased significantly (p<0.01) than control. The average stomach diameter of Group–VI (7.50µg/g B.W NaF) was 5.58±0.35 mm which was decreased significantly (p<0.01) than control.

The current study aimed at observing the toxic effects of commonly used, industrial/pesticidal products containing different concentrations of NaF on albino male mice organs. It shows clearly how NaF aggregates in the liver and various other reproductive and endocrine systems [17-19]. The teeth, bones, and stomach all showed signs of fluoride poisoning; the frequency and degree of these alterations were correlated with the amount of NaF used and the length of exposure [20]. NaF did not change the incidence of preneoplastic and neoplastic lesions at any location in rats of either sex, despite abundant indications of toxicity. This study's findings suggest that NaF is not carcinogenic [20]. The kidney is the organ most important to an animal's ability to withstand prolonged exposure to fluoride [21]. F− excretion in urine is less common in those with renal failure [22]. Even at the typical permissible limits of F in drinking water, they are at significant risk of fluorosis and have increased plasma F levels compared to normal healthy individuals [23]. Animals including humans, have changes in kidney function when exposed to fluoride through their drinking water [24]. Renal tubular injury is indicated by N-acetyl-β-D-glucosaminidase (NAG) and α-glutathione-S-transferase (α-GST) [25]. Studies of F-exposed animals and humans have shown increased levels of NAG and α-GST in urine [26]. It has also been demonstrated that F causes significant histological and ultrastructural alterations in the kidneys of mice and rabbits [27]. A dose-response association between renal tissue damage and fluoride has been observed by researchers. As the kidneys play a major role in eliminating ingested F from the body, decreased renal function raises blood F levels by decreasing urine F excretion [28, 29]. Between 75 and 90 percent of fluoride that is consumed is absorbed. Up to 40% of the fluoride that is swallowed is absorbed from the stomach as hydrogen fluoride (HF), which is created when fluoride is transformed to HF in an acidic stomach. Elevated pH in the stomach reduces the concentration intake of HF, which in turn reduces gastric absorption. The colon absorbs fluoride that is not absorbed in the stomach and is unaffected by pH there [30]. Fluoride is regarded as a

### Table 2: The effects of sodium fluoride and fresh orange juice on albino male mice Stomach and Liver for 30 days

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (Grams ± S.E)</td>
<td>29±1.58</td>
<td>27±1.00</td>
<td>26.67±1.02</td>
<td>25±0.57</td>
<td>23.3±1.00</td>
<td>21±1.02</td>
</tr>
<tr>
<td>Stomach Length (mm ± S.E)</td>
<td>13.2±1.65</td>
<td>12.2±0.59</td>
<td>12.07±1.38</td>
<td>11.53±0.48</td>
<td>11.48±0.35</td>
<td>11.47±0.46</td>
</tr>
<tr>
<td>Stomach Diameter (mm ± S.E)</td>
<td>7.07±0.71</td>
<td>6.33±0.71</td>
<td>5.99±1.34*</td>
<td>5.91±0.50*</td>
<td>5.63±0.45**</td>
<td>5.58±0.35**</td>
</tr>
<tr>
<td>Hepato-Somatic Index (mg ± S.E)</td>
<td>4598.95±98.85</td>
<td>4591.47±91.07</td>
<td>4705.18±68.06</td>
<td>4601.75±390.15</td>
<td>4598.95±98.65</td>
<td>4504.72±309.33</td>
</tr>
<tr>
<td>Gastro-Somatic Index (mg ± S.E)</td>
<td>1914.23±20.02</td>
<td>1816.73±20.52</td>
<td>1816.93±68.59</td>
<td>2055.77±27.24</td>
<td>210.88±142.08</td>
<td>2293.91±230.33</td>
</tr>
</tbody>
</table>

Asterisks indicate significant difference against control **=(p<0.01) and *= (p<0.05)

### DISCUSSION

The effects of sodium fluoride and fresh orange juice on albino male mice stomach and liver for 30 days.
dangerous substance. It has been demonstrated in this context that high fluoride concentrations in extremely acidic media can cause gastric lesions, most likely by encouraging acid back-diffusion to the mucosa [31]. The gastrointestinal tract’s organs are the ones that fluoride exposure negatively affects the most in the body [32]. Fluoride salts have the ability to react with hydrochloric acid (HCl) in the stomach to produce hydrofluoric acid (HF), which can diffuse past the gastric mucosa more easily. Numerous studies in related scientific literature have documented detrimental effects on blood chemistry following consumption of fluoride-enriched water. According to certain research publications, nitric oxide (NO) generation in the blood is boosted when fluorides are taken orally at a dosage of 20 mg/kg [33]. There is proof in scientific literature that long-term fluoride exposure causes oxidative stress and activates lipid peroxidation (LPO) [4]. Due to increased lipid peroxidation and DNA damage in the gastric mucosa, oxidative stress has been linked to the onset of inflammatory bowel disease, peptic ulcers, and gastrointestinal malignancies [6, 34]. In a study it was found that high range of fluoride ions i.e. 150-600 ppm gave way to stress in many important organs especially the heart and kidneys. This included a heightened expression of nuclear kappa factor beta (NF-kB) [35]. Organs of the Group-II (5.00µg/g B.W. of sodium fluoride for 30 days and next 30 days treatment by fresh orange juice) and Group-III (7.50µg/g B.W. of sodium fluoride for 30 days and next 30 days treatment by fresh orange juice) were normal according to the morphology but on the base of morphometric analysis the body weight, hepatic somatic index, gastric somatic index, stomach length and diameter, renal somatic index, (Figure 1 and 2). Organs of Group-IV (2.50µg/g B.W. of sodium fluoride) showed abnormalities the spot on the liver, stomach, (Fig 1 and 2). Organs of Group-V (5.00µg/g B.W. of sodium fluoride) and Group-VI (7.50µg/g B.W. of sodium fluoride) showed morphological abnormalities, blackish color spots on the liver, stomach, liver (Figure 1). The more difference on the base of morphometric analysis between control and these treated groups.

**CONCLUSIONS**

As a conclusion it can clearly be inferred that the oral administration of NaF has highly toxic attributes, when accumulated in high concentration in albino male mice especially liver and stomachand that orange juice can have healing effects on organs affected by NaF toxicity. Hence, it is suggested that sodium fluoride usage should be monitored in humans especially during sensitive periods such as when a mother is expecting and afterwards when babies start feeding on their mother’s milk as it is during the developmental periods that this chemical has the most destructive affects.

**Authors Contribution**

Conceptualization: AA  
Formal analysis: AUMF, AA, AUR, ML  
Writing-review and editing: AUMF, FA, HF, RB, SK  
All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest**

The author declares no conflict of interest.

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**REFERENCES**


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