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Effect of Sodium Fluoride on Glycemic Index and Liver Functions in Rats

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Abstract: From a health standpoint, fluoride (F) is a vital element for humans. It had harmful effects on numerous organs when consumed in high dosages. Fluoride poisoning has been linked to liver damage. The purpose of this study was to see how sodium fluoride (Naf) affected liver function and the glycemic index in adult male albino rats. Fourteen (14) adult male Wistar albino rats were randomly and evenly divided into two groups and given the following treatments for thirty (30) days: G1 Group (Control group), were given distilled water and fed a balanced diet, G2 rats were administered water that contained 100 ppm Naf. The animals were fasted for 8-12 hours before being anesthetized and blood samples were taken by heart puncture technique at zero day (zero time) and (30) day. The following parameters were measured using the serum. The glycemic index contains (glucose, insulin, and insulin resistance), as well as liver function tests such as serum activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT), as well as direct, indirect and total bilirubin concentration. The livers and pancreas were quickly delivered, meticulously dissected out, prepped, and viewed under a light microscope. The results demonstrated that 30 days of exposure to Naf in drinking water produced liver damage manifested by significant increases in blood ALT, AST, and GGT activity as well as significant elevation in serum bilirubin (direct, indirect and total) concentration compared to control group. A significant rise in blood glucose levels and a fall in blood insulin levels and IR were detected at the end of the experiments in Naf treated group when compared to the control. Histopathological changes in Naf treated group was observed in hepatic and pancreatic tissue manifested by generalised degeneration and necrosis of hepatocytes, in addition, to severe degeneration of acinar and pancreatic islet cells. In conclusion, the findings of this investigation revealed Naf therapy-induced liver damage and a change in the glycemic index in adult male rats.

Key words: Sodium fluoride, liver dysfunction, pancreas dysfunction, AST, ALT.

Introduction

Fluorine is the most electronegative element. This element interacts in the form of flouride with several elements and generates ionised fluorides that can accumulate in the body due to its relatively high electronegativity (Mittal and Flora, 2006). Fluoride element is a mineral that is found everywhere and observed in tiny levels in soil, water, plants, animals and is a widespread air contaminant in a variety of industrial operations (Whitford and Stipanuk, 2000). Ionised fluorides have been implicated in the clinical manifestations of fluoride poisoning, including tissue and organ damage (Eraslan et al*.,* 2007; Mittal and Flora, 2006). Moderate F intake promotes bone formation and is beneficial for caries prevention. Ingestion or inhalation of high F dosages, on the other hand, has negative impacts on human and animal health (Lech, 2011; Zhou et al*.,* 2012). F poisoning affects more than just the bones and teeth (Fawell

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et al., 2006), but also soft tissues such as the kidney (Shashi et al., 2002), brain (Shashi, 2003), and blood (Karadeniz and Altıntas, 2008), peripheral blood (Lu et al*.,* 2017), and the spleen and kidney of mice (Kuang et al., 2017; Luo et al., 2017). According to one study, fluoride stimulates the development of bone cancer (Lowry et al., 1951) and accelerated the ageing of the human body (Mokrzynskei et al*.,* 1994). Many scientific studies unequivocally stated that fluoride intake was excessive and/or prolonged causing oxidative stress. Increased lipid peroxidation and reduced antioxidant enzyme levels in soft tissues such as the liver, kidney, brain, lung, and testes by creating reactive oxygen species (ROS) and free radicals, which were thought to be a major cause of intoxication (Abdel-Gawad et al*.,* 2014; Zhang et al*.,* 2014). The current study looks at the effect of Naf on biochemical indicators related to liver function and the glycemic index.

Experimental Design

Albino male Wistar rats (aged 10-11 weeks and weighing 280 g) were utilised throughout this investigation. Rats were used at all stages of the research in plastic cages in a climate-controlled room (23-25°C) from June 2020 to July 2020, delivering 12 hours of daily light (7 a.m. to 19 p.m.) and a 12-hour night cycle. They were kept at the animal home of the University of Baghdad's College of Veterinary Medicine for 10 days to acclimate to the experimental settings. During the experiment, rats were fed a regular pellet meal and given sufficient access to water. Fourteen (14) albino rats had been split into two equal groups and given the following treatments every day for thirty (30) days: Male rats in Group G1 were given distilled water; group G2 male rats received 100 ppm drinking water with sodium fluoride (Sigma-Aldrich chemical company). Fasting blood samples were taken on day zero and at the end of the experiment (30 days), blood was drawn by heart puncture technique, and rats were sedated by I/M injection of Ketamine 90 mg/kg and Xylazine 40 mg/kg. Serum was prepared by centrifuging the sample (blood) for 15 minutes at 3000 rpm and determined the parameters listed below: Glycemic index including (serum glucose concentration, serum insulin concentration and insulin resistance), (ALT) and (AST) activity using ALT and AST kit (Redox, United Kingdom), gamma glutamyl transferase enzyme (GGT) activity using GGT kit (Biosystem, Spain); direct, indirect and total bilirubin concentration by using an enzymatic kit (Biosystem, Spain). For measuring weight gain the body weight of animals was measured at the beginning of the experiment and then weekly until the end of the experiments. Section from the liver and pancreas tissues were taken from each experiment animals for histopathological study according to Suvarna et al*.* (2013). The tissues were prepared for regular histological evaluation by trimming and processing them. They were then soaked in paraffin wax and chopped into 4-5 sections. Hematoxylin and eosin staining was used to stain all tissue slices. A light microscope was used to examine tissue slides at the University of Baghdad's Department of Pathology, Faculty of Veterinary Medicine. The Statistical Assessment System- SAS (SAS, 2012) application was used to determine the influence of various components in research parameters. In this investigation, the T-test was utilized to compare means that were statistically significant (Snedecor and Cochran, 1973).

Results and Discussions

Clinical Observations

Throughout the investigation, there have been no discernible variations in the animals' appearance or behaviour. At the conclusion of the research, no one died in any of the experimental groups. However, there is a decrease in appetite.

Body Weight

According to the results as tabulated in Table 1, the fluoride therapy group's body weight did not influence significantly; however, as compared to the control (G1) group, the group (G2) exhibited a significant decrease ($P \leq 0.05$) in body weight gain.

Table 1: Effect of sodium fluoride on initial, final body weight and body weight gain in adult male rats

Groups	*Mean ± SE*		
	Initial weight (gm.)	*Final weight (gm.)*	*Weight gain (gm.)*
G1: Control	270.57 ±2.96	275.28 ±2.83	4.71 ±1.63
G2: Naf	271.00 ±5.82	265.00 ±6.17	-6.00 ±1.59
T-test	14.22 NS	14.79 NS	0.050*

* ($P \leq 0.05$), NS: Non-Significant.

The results are shown as mean SE, with n=7 from each group. G1: Rats in the control group were given distilled water; G2: For 30 days, animals were given 100 ppm sodium fluoride in their drinking water.

Fluoride enters the body through drinking water as

the main route of chronic exposure. Fluoride was thus delivered to rats in the same way in the current study (Al-Sabaawy and Al-Kaisie, 2021). Animals in group G2 gained less weight than those in group G1. Fluoride exposure reduced body weight gain significantly ($P \leq 0.05$), this might be related to atrophic gastritis and poor gastrointestinal absorption, reduced hunger and disrupted nutrient digestibility, which can finally lead to excessive breakdown of cellular macromolecules, resulting in weight loss (Das et al*.,* 1994). A reduction in animal appetite was found in the Naf group when compared to the control group, and the results were reported (AL-Chalabi, 2014).

Glycemic Index

When compared to the control group, animals given Naf for 30 days had a significant ($P \leq 0.01$) increase in serum glucose level (212.30 ±2.32) as well as a significant ($P \leq 0.05$) reduction in insulin and insulin resistance (1.228 ±0.09, 0.637 ±0.04) (Table 2).

The results are shown as mean SE, with n=7 from each group. G1: Rats in the control group were given distilled water; G2: For 30 days, animals were given 100 ppm sodium fluoride in their drinking water.

Diabetic characteristics of Naf poisoning are indicated by increased blood serum glucose levels. The following are some of the mechanisms that could be at work in an elevated blood sugar level: a) decreased pancreatic insulin release, b) significant changes in carbohydrate metabolism by inhibiting key enzymes are involved in glycolysis and the TCA cycle, and c) hypomagnesaemia, which may worsen insulin resistance, a situation that frequently precedes hyperglycemia, or it may be the result of insulin resistance (Dousset et al*.,* 1987; Simmons et al*.,* 2010). Following NaF intoxication, the current study's findings were consistent with the previous findings. Exposure to Naf significantly lowers the actions of pancreatic lipase, protease and alpha-amylase, thereby causing damage to the structures of digestive enzymes (Mulimani and Gopal, 1989; Zhan et al*.,* 2005). Impaired glucose metabolism is a significant indicator of hepatotoxicity. The current study found that rats fed with NaF for four weeks had a significant decrease in insulin levels and an increase in blood sugar levels. Previous research yielded similar results (Menoyo et al*.,* 2005; Rigalli et al*.,* 1995). The observed hyperglycemia caused by a reduction in insulin production could be part of the explanation for NaF. Sodium fluoride administration has been proven to block insulin secretion in rats and humans, resulting in reduced insulin plasma levels (Rigalli et al*.,* 1995). It also influences insulin secretion by changing the intracellular signaling system involved in insulin secretion (25). F levels were found to be linked to b-cell malfunction (Menoyo et al*.,* 2008). B-cell failure is linked to the release of entire and partially processed proinsulin insulin precursor (Yoshioka et al*.,* 1988). Furthermore, F has been shown to reduce pancreatic tissue sensitivity to glucose stimulation (Menoyo et al*.,* 2008).

Liver Function Biomarkers

Conclusively, the data from Table 3 demonstrated a substantial ($P \leq 0.01$) rise in serum activity of ALT, AST and GGT in the sodium fluoride group in contrast to the control group.

The results are shown as mean SE, with n = 7 from each group. G1: Rats in the control group were given distilled water; G2: For 30 days, animals were given 100 ppm sodium fluoride in their drinking water.

The results are shown as mean SE, with n=7 from each group. G1: Rats in the control group were given distilled water; G2: For 30 days, animals were given 100 ppm sodium fluoride in their drinking water.

The data in Table 4 demonstrated the influence of NaF on direct, indirect and total bilirubin concentrations, at the end of the study; the Naf group had a significant ($P \leq 0.01$) elevation in comparison to the control group.

The current study's findings show that the NaF group has decreased liver function, as evidenced by increased

Table 2: Effect of sodium fluoride on glycemic index (glucose, insulin and insulin resistance) in adult male rats

Groups	*Mean ± SE*					
	Glucose (mg/dl)		*Insulin concentration (µlU/ml)*		*Insulin resistance (IR)*	
	Zero time	*30 day*	*Zero time*	*30 day*	*Zero time*	*30 day*
G1: Control	91.83 ±-0.40	91.35 ±0.41	3.57 ±0.15	3.53 ±0.17	0.804 ±0.03	0.791 ±0.04
G2: Naf	91.17 ±0.39	212.30 ±2.32	3.70 ±0.20	1.228 ±0.09	0.827 ±0.04	0.637 ±0.04
T-test	1.229 NS	5.13**	0.561 NS	0.431*	0.123 NS	0.134*

* ($P \leq 0.05$), ** ($P \leq 0.01$), NS: Non-Significant.

Table 3: Effect of sodium fluoride on the liver function (serum activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase activity (GGT) in adult male rats

Groups	*Mean ± SE*					
	ALT (U/L)		*AST (U/L)*		*GGT (U/L)*	
	Zero time	*30 day*	*Zero time*	*30 day*	*Zero time*	*30 day*
G1: Control	4.55 ±0.18	4.47 ±0.14	6.66 ±0.51	7.27 ±0.49	23.82 ±0.79	24.35 ±0.73
G2: Naf	4.65 ±0.12	54.60 ±2.48	6.32 ±0.71	44.54 ±1.20	23.22 ±0.54	49.32 ±0.56
T-test	0.480 NS	5.424**	1.903 NS	2.828**	2.099 NS	2.007**

** ($P \leq 0.01$), NS: Non-Significant.

Table 4: Effect of sodium fluoride on direct, indirect and total bilirubin concentration in adult male rats

Groups	*Mean ± SE*					
	Direct bilirubin (mg/dl)		*Indirect Bilirubin (mg/dl)*		*Total Bilirubin (mg/dl)*	
	0 time	*30 day*	*0 time*	*30 day*	*0 time*	*30 day*
G1: Control	0.134 ±0.004	0.130 ±0.004	0.474 ±0.022	0.532 ±0.019	0.608 ±0.022	0.662 ±0.018
G2: Naf	0.125 ±0.003	0.710 ±0.127	0.478 ±0.028	0.778 ±0.053	0.604 ±0.031	1.527 ±0.112
T-test	0.0114 NS	0.278**	0.0782 NS	0.124**	0.0825 NS	0.2492**

** ($P \leq 0.01$), NS: Non-Significant.

liver enzyme parameters in the blood, particularly AST, ALT and GGT, which are indirectly hepatocytes. It is well established that an increase in these markers implies hepatocellular injury (Bulle et al*.,* 1990). The average increase in these markers might be a secondary occurrence following NaF-induced LPO of hepatocyte membranes, with a sharp rise in biomarker leakage from the liver tissue. LPO of cell membranes results in membrane fluidity reduction, membrane potential alterations, and elevated membrane permeability (Nehru and Anand, 2005), all of which result in enzyme leakage from liver cells. Previous research supported a significant rise in ALT and AST levels seen in mice and rats following Naf-intoxication in a dose-dependent pattern (Trivedi et al*.,* 2008; Xiao-Ying et al*.,* 2003). The rise in serum direct, indirect, and total bilirubin concentrations represented the severity of hepatotoxicity (Lin et al*.,* 1997).

Histopathological Results

Comparing to Figure 1 that showed the normal histological structure of the liver in control groups, there are sections of the sodium flouride group (G2) that showed generalised vacuolar degeneration and necrosis of hepatocytes and marked intra vascular hemolysis (Figures 2 and 3). While the section in the pancreas on the G2 group showed severe degeneration of pancreatic islet cell and acinar cells (Figure 5)

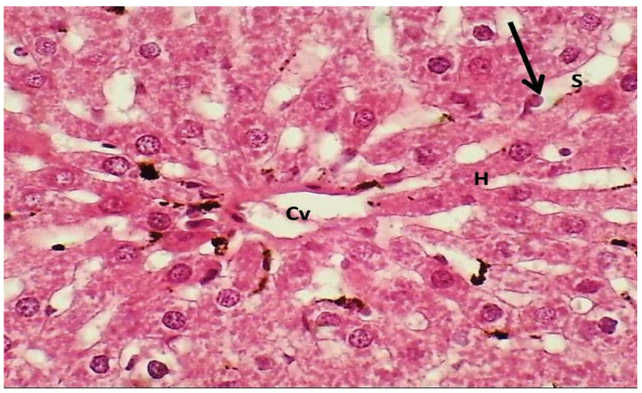

Figure 1: Magnified section of liver lobule (Control) shows: Central vein (Cv), hepatocytes (H), sinusoids (S) and Kupffer cells (Arrows). H&E stain (400×).

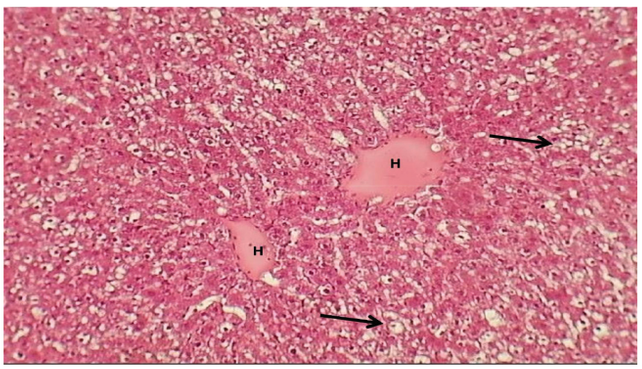

Figure 2: A liver segment (Group of Sodium Fluoride) is shown. Generalised degeneration and necrosis of hepatocytes (arrows) and intravascular hemolysis (H). H&E stain (100×).

compared to the G1 group (Figure 4). In the current investigation, the histopathological lesions in the liver sections of rats given Naf are consistent with the biochemical results described herein, indicating fluoride poisoning (Panneerselvam et al., 2013; Parihar et al., 2013; Sewelam, 2017) that could be attributed to ROS generation by Naf (Al-Saadet al., 2021; Johar et al., 2004). Pandey et al. (2008) hypothesised that F-induced hepatocyte necrosis was caused by glutathione depletion and oxidative stress in these cells, which resulted in apoptosis, swelling of cell organelles, particularly the mitochondria and rough endoplasmic reticulum, and lysosomal disintegration, with subsequent necrosis, shrinkage, and nuclear dissolution of hepatocytes. Previous literature (Shashi et al., 2010; Stawiaska- Pieta et al., 2008) showed that fluoride poisoning results in a loss of selective permeability of the cell membrane and dilatation of the cytoplasmic component as a result of intracellular fluid redistribution (Agha et al., 2012; Gutowska et al., 2011; Stawiarska-Pieta et al., 2009) relieved the occurrence of inflammatory cell reactions involving macrophage and their differentiation, such damaging effect of the pancreas could be due to oxidative stress induced by Naf.

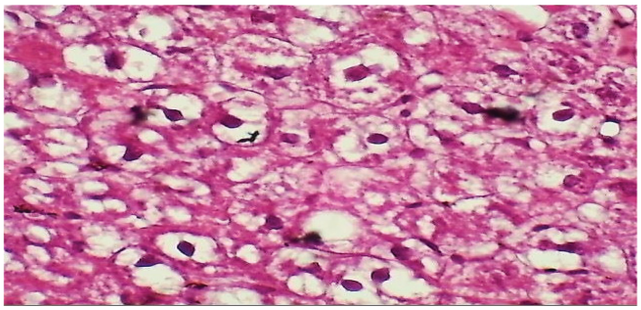

Figure 3: A portion of the liver (Group of Sodium Fluoride) is shown.Vacuolar degeneration and necrosis of hepatocytes. H&E stain (400×).

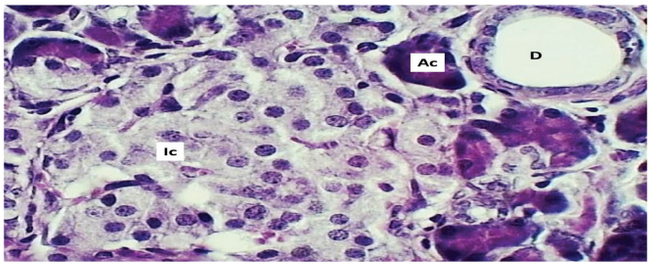

Figure 4: The pancreatic section (Control group) demonstrates: normal appearance of islet cells (Ic), acinar cells (Ac) and duct (D). H&E stain (400×).

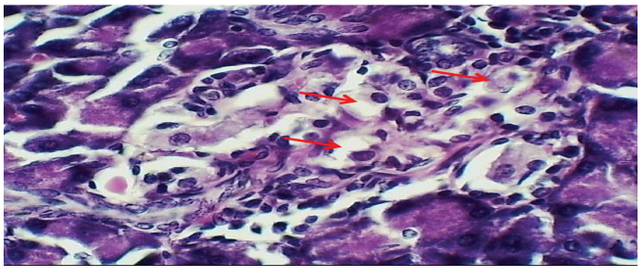

Figure 5: The pancreatic section (group of sodium fluoride) demonstrates: severe degeneration of pancreatic islet cells (arrows) and acinar cells. H&E stain (400×).

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