

# STUDYING THE AMELIORATIVE EFFECT OF CURCUMIN ON THE PANCREAS, IMMUNITY, AND OXIDATIVE STRESS OF SODIUM FLUORIDE INTOXICATED MALE ALBINO RATS

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## ABSTRACT

**Background:** Egypt is one of the top 21 countries having problems with endemic fluorosis. High fluoride permeability allows F ion to penetrate cell membranes and accumulates in diverse soft tissues. Although fluorosis has been investigated for many years, there are relatively few studies of its effect on the digestive system such as the pancreas. The relationship between fluoride and immunity is an ongoing topic of discussion as fluoride impairs the immune system's functions. Curcumin is known to have anti-inflammatory, antioxidant, and immunomodulatory effects. **Objectives:** This work aimed to study the subacute toxicity of Sodium Fluoride (NaF) on the pancreas, immunity, oxidative stress, and the probable ameliorative effect of curcumin. **Material and methods:** the study was conducted on 60 mature male albino rats, they were randomly divided into: Group I (Negative control), Group II (Curcumin group): Rats received curcumin (15.75 mg/kg) by oral gavage daily, Group III: rats daily received NaF (10 mg/kg) by oral gavage, group IV: rats received NaF (10mg/kg/day) by oral tube concomitantly with curcumin (15.75mg/kg) daily, Group V: Rats daily received NaF (25mg/kg) by oral gavage, and Group VI: Rats received NaF (25mg/kg) by oral gavage concomitantly with curcumin (15.75 mg/kg) daily. The study was carried out for four weeks. In the end, all rats were sacrificed and blood was collected to assess the measurements of insulin, glucose, Immunoglobulin M, Immunoglobulin G, Malonaldehyde (MDA) and total antioxidant capacity TAC levels. The pancreas was preserved for histopathological examination by light microscope. **Results:** The present study revealed a statistically significant decrease ( $P < 0.001$ ) in the insulin, IgG, IgM, and TAC levels associated with a statistically significant increase ( $P < 0.001$ ) in glucose, and MDA levels in NaF only treated groups. On the other hand, there were statistically significant increases ( $P < 0.001$ ) in the insulin, IgG, IgM and TAC levels associated with significantly significant decreases ( $P < 0.001$ ) in glucose and MDA levels in curcumin treated groups. The pancreatic tissue showed marked histological changes with apparent improvement with curcumin. **Conclusion:** NaF has toxic effects on pancreatic parameters, immunity, and oxidative stress. Curcumin has a protective role against such harmful effects.

**Keywords:** sodium fluoride, curcumin, pancreas, immunity, oxidative stress

## INTRODUCTION

Fluorosis is an endemic and international issue that affects many continents. The prevalence of fluoride-related health issues in people has been significantly impacted by the growth in environmental pollution brought on by growing industrialization. Worldwide, elevated fluoride levels in water used for drinking have been connected to potential health concerns

(Demarchi et al., 2023). Easily found online as a 98% pure reagent, Sodium fluoride (NaF) is a colourless and odourless granules that finds applications as a DIY toothpaste, pesticide, and wheel cleaner (Bridwell et al., 2019). Fluoride binds with many proteins, specially the enzymes inhibiting their activity (Waugh, 2019). Superoxide anions production, Mitochondrial

toxicity (*Fujii et al., 2022*), One of the ways that sodium fluoride causes toxicity is through inhibiting the migration of specific cell types, including sperm as well as embryonic neurons (*Guth et al., 2020*). The overall decrease in insulin serum level associated with high blood glucose is due to the ability of fluoride to reversibly inhibit insulin Trimeric has long been used as an ingredient and spice in meals. Due to its distinctive yellow colour, flavour, and antioxidant potential, it is frequently used to improve the palatability and storage durability of foods (*Latoch et al., 2023*). Curcumin has a beneficial anti-diabetic effect by reducing the production of superoxide and inhibiting vascular protein kinase C, which improves diabetes-induced endothelial dysfunction. It's interesting to note that the recent researches have shown that curcumin can directly quench oxygen free radicals that can produce oxidative damage (*Zhang and Kitts, 2021*). Tumor Necrosis Factor Alpha (TNF $\alpha$ ) is involved in the development of several inflammatory disorders through activation of Nuclear factor- $\kappa$ B (NF- $\kappa$ B). Curcumin inhibits both the simultaneous activation of NF- $\kappa$ B and TNF production (*Radzka et al., 2023*). By eliminating oxygen free radicals and preventing lipid peroxidation, curcumin possesses strong antioxidant qualities (*Mathew et al., 2015*). Although fluorosis has been investigated for many years, there are relatively few studies of its effect on the digestive system such as the pancreas. The subsequent study seeks to determine the potential restorative impact of curcumin while also examining the subacute toxicity of sodium fluoride on the pancreas, immunology, and oxidative stress biomarkers malondialdehyde (MDA) and total antioxidant activity (TAC) following numerous oral dosages.

## MATERIAL AND METHODS

### I- DRUGS AND CHEMICALS

- Sodium Fluoride (NaF)  $\geq$  98% (G. Frederick Smith (GFS) chemicals company, India).
- Curcumin 98% (C<sub>21</sub>H<sub>20</sub>O<sub>6</sub>–Techno chemicals, India) was macerated in distilled water and filtered (*AbdEllah et al., 2021*).

- ELISA kits to measure insulin level were purchased from CORTEZ diagnostics company.

- On call plus blood glucose test strips were purchased from private pharmacy.

- 100 microns of serum were measured quantitatively using Kits to measure IgG level and IgM level supplied from Roche company diagnostics (Indianapolis, IN, USA).

- 200 microns of serum were measured quantitatively using a kit of oxidative stress biomarkers (MDA and TAC) supplied from Biodiagnostic company-Egypt.

### II- APPARATUSES

- UV 2300 spectrophotometer (USA): It is a Split Beam Scanning UV-Vis spectrophotometer which has a wave length range of 190-1100 nm and comes with a programmable 5-turret sample compartment. It was used for measurement of MDA and TAC, Present in Clinical Pathology Laboratory in Sohag University Hospital.

- Cobas e 411UV 2300: is a fully automatic autoanalyzer applied to the immune analysis, whose measurement basis is Electrochemiluminescence (ECL). ECL technology uses streptavidin-coated magnetic microparticles as a solid phase, antigen/antibody interactions, and interference suppression methods. It was used for measurement of serum levels of insulin hormone, IgG and IgM. Present in Clinical Pathology laboratory in Sohag University Hospital.

- Electronic glucometer, On Call Plus-model (12-GM-GM-NC-PL): it was used for measurement of blood glucose level. It was purchased from private pharmacy.

### III-ANIMALS

Sixty mature male albino rats weighing between 180 and 220 grams were acquired from and kept at the Animal House of Faculty of Medicine, Sohag University, Egypt. One week prior to the study, they were kept in sterile polypropylene cages with stainless grill tops with surrounding temperature, 18-24 °C in order to acclimatize to their new environment. The study was conducted in adhering to the guidelines for the use and care of laboratory

animals, which were authorized by the Sohag University Faculty of Medicine's Medical Research Ethics Committee. In June 2022, the study was conducted.

### **EXPERIMENTAL DESIGN**

Rats were divided into 6 groups randomly; each group consisted of 10 animals. The study lasted for 4 weeks.

**Group I (Negative control group) (NC):** was provided with distilled water and basic diet.

**Group II (Curcumin treated group) (Cur):** was treated daily with curcumin (15.75 mg/kg) by oral gavage (*Hashem et al., 2011*).

**Group III (T10)** was treated with NaF (10 mg/kg) (1/25 of the oral LD50 value in rats) by oral gavage daily (*Labib et al., 2021*).

**Group IV (T10 + Cur):** received NaF (10 mg/kg.) concomitantly with curcumin (15.75 mg/kg) daily.

**Group V (T25):** received NaF (25 mg/kg) (1/10 of the oral LD50 value in rats) daily by oral gavage.

**Group VI (T25 + Cur):** Received NaF (25 mg/kg) by oral gavage concomitantly with curcumin (15.75 mg/kg) daily.

### **STATISTICAL ANALYSIS**

Data were presented in tables as mean  $\pm$  standard deviation of the mean ( $M \pm SD$ ). Statistical analysis was performed using SPSS version 16. software and significant differences between groups were calculated using Post-Hoc Tukey test and One way ANOVA where a P-value of  $\leq 0.05$  was considered statistically significant.

## **RESULTS**

### **I- BIOCHEMICAL RESULTS:**

#### **1-Serum level of insulin and glucose:**

Both NaF treated groups (III & V) showed significant statistical decrease in the mean values of fasting serum insulin (0.47  $\mu$ IU/ml) and (0.16  $\mu$ IU/ml) respectively as compared to

negative control group (1.35  $\mu$ IU/ml) and curcumin treated (1.12  $\mu$ IU/ml) ( $p$  – value 0.0001) associated with significant statistical increase in the mean values of fasting blood glucose (108 mg/dl) and (124.5 mg/dl) respectively as compared to negative control group (86.1 mg/dl) and curcumin treated group (86.4 mg/dl) ( $p$  – value 0.0001) **Table (1)& Graph (1,2)**. While, curcumin treated groups (IV & VI) showed significant statistical rise in the mean value of fasting serum insulin (1.26  $\mu$ IU/ml) and (1.1  $\mu$ IU/ml) respectively as compared to groups III (0.47  $\mu$ IU/ml) and group V (0.16  $\mu$ IU/ml) ( $p$  – value 0.0001). There were significant statistical decreases in the mean values of fasting blood glucose of both groups IV (88.5 mg/dl) and VI (88 mg/dl) as compared to groups III (108 mg/dl) and V (124.5 mg/dl), p-values were (0.0001 and 0.004) respectively **Table (1)& Graph (1,2)**.

#### **2- Serum level of IgG and IgM:**

The mean values of serum IgG of both groups III (216 mg/dl) and V (200.4 mg/dl) significantly decreased statistically as compared to NC (255.3 mg/dl) and Cur. (253.2 mg/dl) groups ( $p$  – value 0.0001) **Table (1) & Graph (3)**. Also, the mean values of serum IgM of both groups III (8.1 mg/dl) and V (5.5 mg/dl) showed significant reduction as compared to negative control group (10.4 mg/dl) and curcumin treated group (10.9 mg/dl) ( $p$  – value 0.0001) **Table (1)& Graph (4)**. Curcumin administration caused significant statistical rise in the mean values of serum IgG of both groups IV (252.8 mg /dl) and VI (254.8 mg/dl) as compared to III (216 mg/dl) and group V (200.4 mg /dl) ( $p$  – value 0.0001). Curcumin also resulted in significant statistical elevation in the mean values of serum IgM of both groups IV (10 mg/dl) and VI (9.6 mg/dl) as compared to group III (8.1 mg/dl) and group V (5.5 mg/dl) ( $p$  – value 0.0001) **Table (1) & Graph (3,4)**.

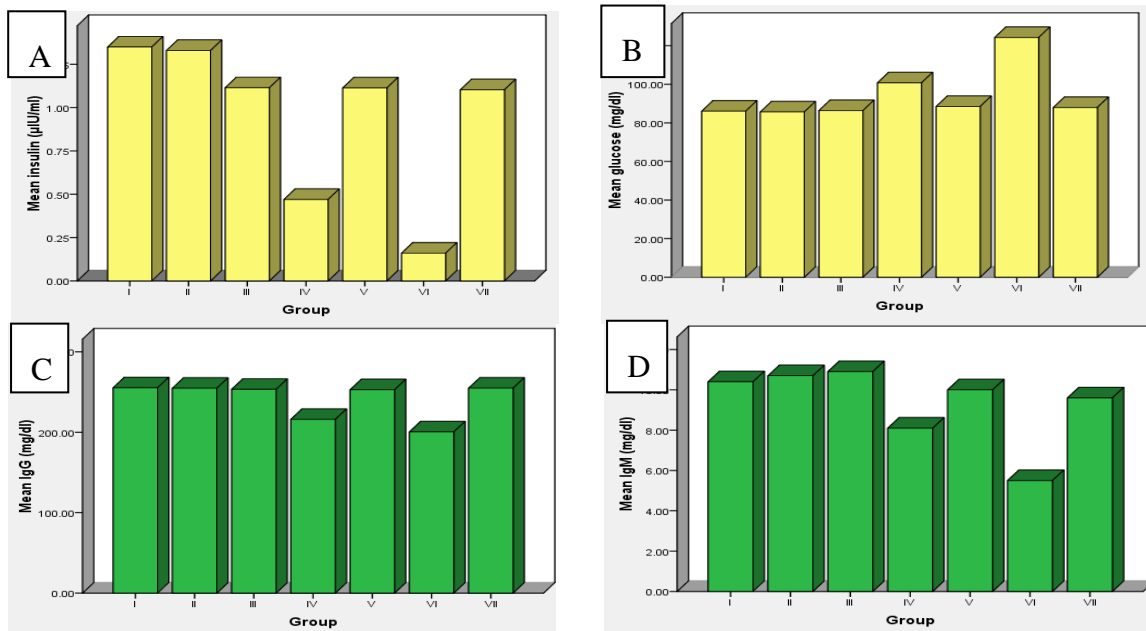
**Table (1):** Comparison of blood levels of insulin, glucose, IgG and IgM among the studied groups

Parameters	Insulin ( $\mu$ IU/ml)	Glucose (mg/dl)	IgG (mg/dl)	IgM (mg/dl)
	Mean $\pm$ SD			
Groups				
Group I	1.35 $\pm$ 0.23 <sup>ce</sup>	86.1 $\pm$ 1.85 <sup>ce</sup>	255.3 $\pm$ 2.5 <sup>ce</sup>	10.4 $\pm$ 1.07 <sup>ce</sup>
Group II	1.12 $\pm$ 0.15 <sup>ce</sup>	86.4 $\pm$ 1.34 <sup>ce</sup>	253.3 $\pm$ 3.5 <sup>ce</sup>	10.9 $\pm$ 0.87 <sup>ce</sup>
Group III	0.47 $\pm$ 0.19 <sup>abde</sup>	108 $\pm$ 2.5 <sup>abde</sup>	216 $\pm$ 3.49 <sup>abde</sup>	8.1 $\pm$ 0.87 <sup>abde</sup>
Group IV	1.26 $\pm$ 0.18 <sup>c</sup>	88.5 $\pm$ 1.58 <sup>c</sup>	252.8 $\pm$ 3.45 <sup>c</sup>	10 $\pm$ 0.81 <sup>c</sup>
Group V	0.16 $\pm$ 0.07 <sup>abcf</sup>	124.5 $\pm$ 2.9 <sup>abcf</sup>	200.4 $\pm$ 3.09 <sup>abcf</sup>	5.5 $\pm$ 0.52 <sup>abcf</sup>
Group VI	1.1 $\pm$ 0.25 <sup>e</sup>	88 $\pm$ 1.4 <sup>e</sup>	254.8 $\pm$ 2.25 <sup>e</sup>	9.6 $\pm$ 1.01 <sup>e</sup>
P – value	0.000**	0.000**	0.000**	0.000**

**SD: Standard deviation**

**\* Significant difference at p-value < 0.05**

**\*\*\* Significant difference at p-value < 0.001** a: significant when compared to group I, b: significant when compared to group II, c: significant when compared to group III, d: significant when compared to group IV, e: significant when compared to group V, f: significant when compared to group VI



**Figure (1):** Graphs showing the following: (A): The mean value of fasting serum insulin ( $\mu$ IU/ml) in the studied groups. (B): The mean value of fasting blood glucose (mg/dl) in the studied groups. (C): The mean value of IgG (mg/dl) in the studied groups. (D): The mean value of IgM (mg/dl) in the studied groups

### 3- Oxidative stress biomarker:

The serum level of MDA showed significant rise as regard its mean value of in of NaF only treated groups III (3.5 nmol/ml) and V (5.5 nmol/ml) as compared to groups I (2.88 nmol/ml) and II (2.94 nmol/ml) . Where p-values were (0.007 and 0.015, 0.0001, and 0.0001) respectively. While the TAC level in the serum significantly dropped NaF only treated groups III (0.049 mM /L) and V (0.024 mM/ L) as compared to groups I (0.0774 mM/L) and III (0.083 mM/L) **Table (2) & Graph (5,6)**. Where p- values were < 0.0001

**Table (2).** Giving curcumin results in significant statistical fall in the mean values of MDA of both groups IV (2.7 nmol/ml) and VI (2.57 nmol/ml) as compared to groups III (3.5 nmol/ml) and V (5.5 nmol /ml) (*p – value 0.0001*) associated with increase that was significant statistically in the mean value of TAC of both groups IV (0.07mM/L) and VI (0.067 mM/L) as compared to groups III (0.049 mM/L) and V (0.024 mM/L) (*p – value 0.0001*) **Table (2) & Graph (5,6)**.

**Table (2):** Comparison of oxidative stress biomarkers (MDA, TAC) among the studied groups

Parameters	MDA (nmol/ml)	TAC (mM/L)
Groups	Mean ± SD	
Group I	2.88 ± 0.33 <sup>ce</sup>	0.077 ± 0.003 <sup>ce</sup>
Group II	2.94 ± 0.33 <sup>c e</sup>	0.082 ± 0.003 <sup>ce</sup>
Group III	3.5 ± 0.47 <sup>abde</sup>	0.049 ± 0.008 <sup>abde</sup>
Group IV	2.7 ± 0.17 <sup>c</sup>	0.07 ± 0.004 <sup>c</sup>
Group V	5.5 ± 0.62 <sup>abcf</sup>	0.024 ± 0.01 <sup>abcf</sup>
Group VI	2.57 ± 0.3 <sup>e</sup>	0.067 ± 0.003 <sup>e</sup>
P – value	0.000**	0.000**

**SD: slandered deviation**

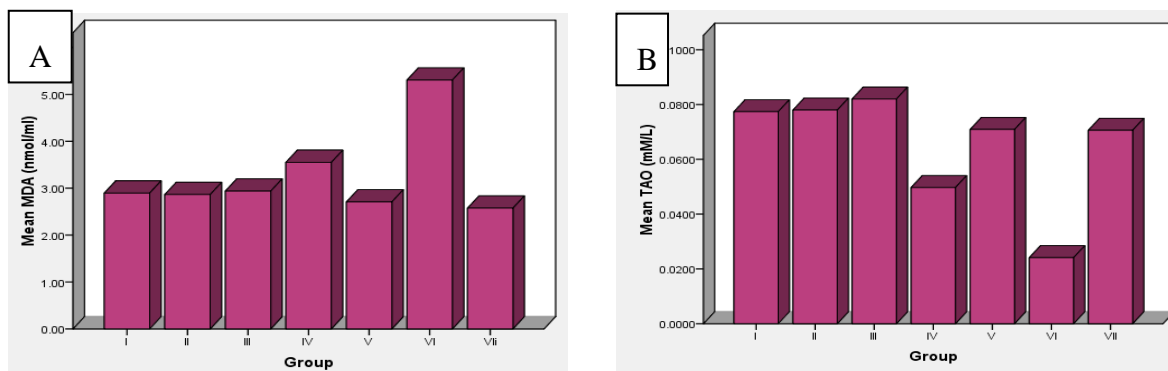
\* **Significant difference at p-value < 0.05**

\*\*\* **Significant difference at p-value < 0.001** a: significant when compared to group I,

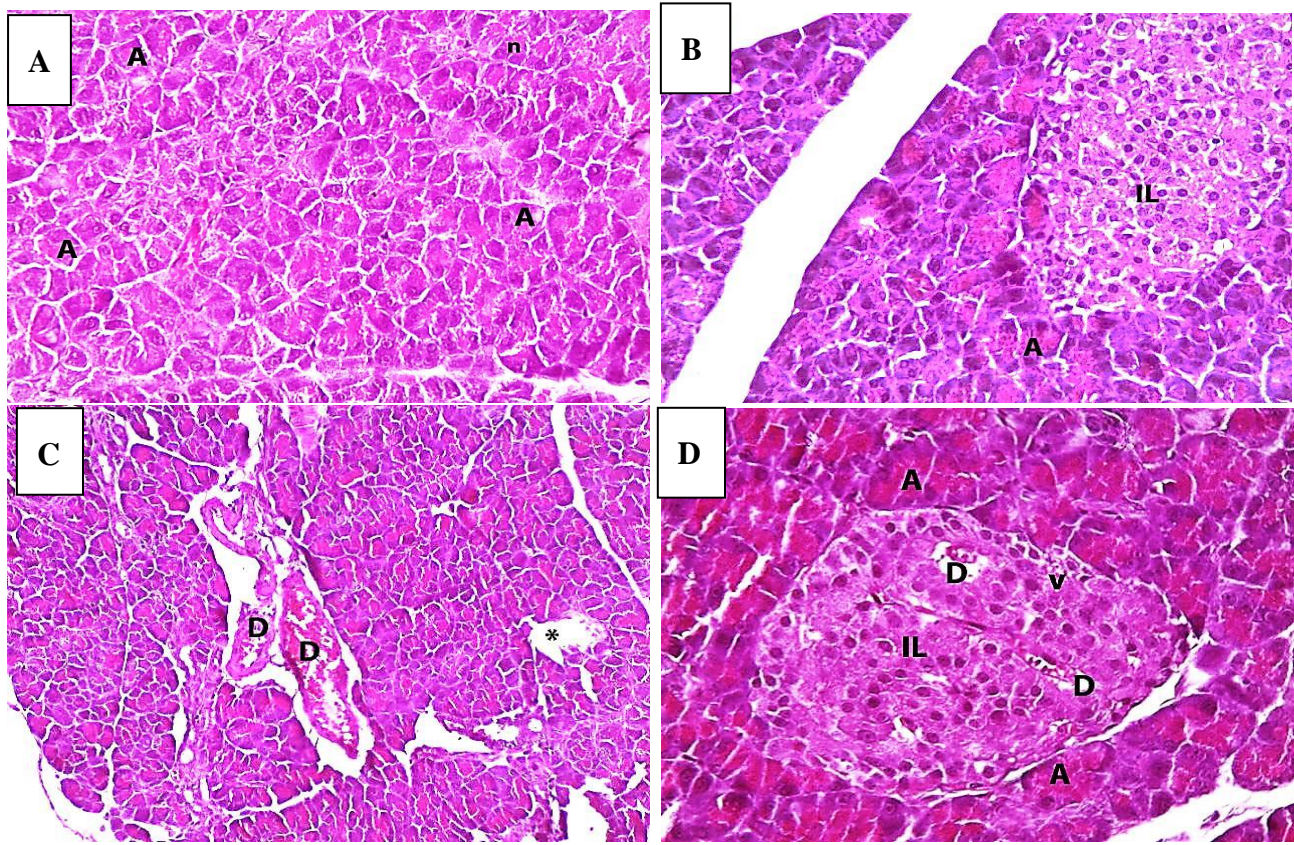
b: significant when compared to group II, c: significant when compared to group III,

d: significant when compared to group IV, e: significant when compared to group V, f: significant

when compared to group VI.



**Figure (2):** Graphs showing the following: (A): The mean value of MDA (nmol/ml) in the studied groups. (B): The mean value of TAC (mM/L) in the studied groups.



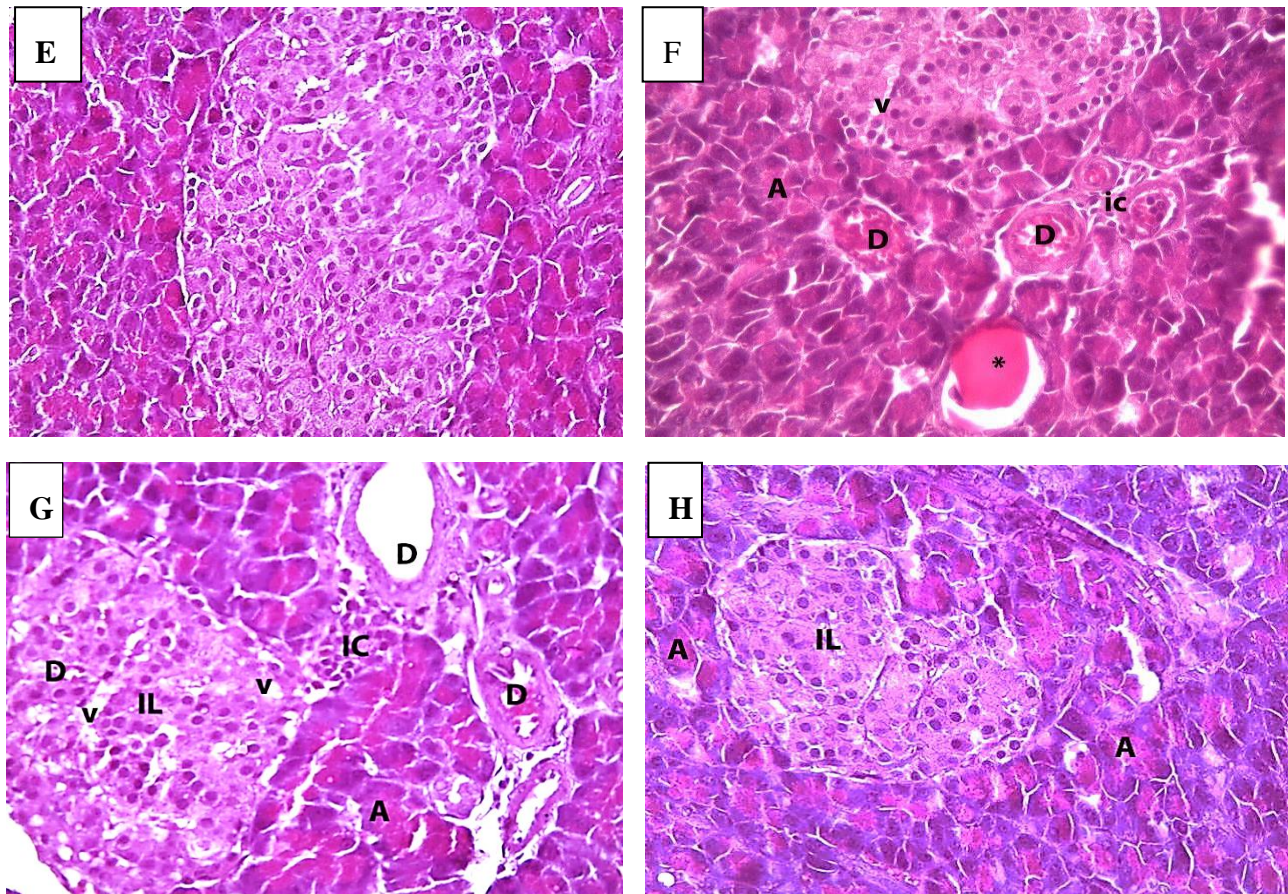
**Figure (3):** Histological changes of the pancreas of different studied groups

(A): A photomicrograph of control pancreas showing; pancreatic lobules consisting of multiple pancreatic acini (A) separated by thin c.t. septa. Each acinus contains few cells with vesicular nucleus (n) and basal basophilic cytoplasm, and apical acidophilic zymogen granules (Hx &E X400).

(B): A photomicrograph of G II(Cur) adult pancreas showing; pancreatic lobules consist of multiple pancreatic acini (A). Each acinus contains few cells with vesicular nucleus and basal basophilic cytoplasm, and apical acidophilic zymogen granules. Definite pale area (IL) of discrete cells and multiple blood capillaries in between (lets of langerhans) (Hx &E X400)

(C): A photomicrograph of pancreas of group (III) showing; pancreatic lobules consist of multiple pancreatic acini. Multiple congested dilated blood vessels (D) and degenerated acini (\*) (Hx & E X200).

(D): A photomicrograph of pancreas of group III (T10) animals showing; pancreatic lobules consist of multiple pancreatic acini (A) and islet of langerhans (IL). Dilated congested blood capillaries are seen within the islet (D) (Hx &E X400).



**Figure (4):** Histological changes of the pancreas of different studied groups

(E): A photomicrograph of pancreas of group IV (T10 + Cur) animals showing; pancreatic lobules consist of multiple normal pancreatic acini and normal islet of langerhans. (Hx & E X 400)

(F): A photomicrograph of pancreas of group V (T25) animals showing; pancreatic lobules consist of multiple pancreatic acini (A) and vacuolated cells of islets of Langerhans (v). Note: Multiple congested dilated blood vessels (D), degenerated acini (star) and inflammatory cells (IC) are seen in the c.t. septa (Hx & E x400).

(G): A photomicrograph of pancreas of group V (T25) animals showing; pancreatic lobules consist of multiple pancreatic acini (A), congested dilated blood vessels (D) and inflammatory cells (IC) are seen in the c.t. septa. Note: Islets of langerhans have dilated blood capillaries (D) and multiple vacuolated cells (v). (Hx & E X400).

(H): A photomicrograph of pancreas of group VI (T25+Cur) animals showing; pancreatic lobules consist of multiple normal pancreatic acini (A) and islets of Langerhans (IL) (Hx & E X 400).

## DISCUSSION

Fluorosis is one of the most significant health risks associated with environmental pollution. Because the levels of fluoride present are frequently higher than permitted

thresholds, fluoride pollution in drinking water is a global issue. Because of its ability to prevent inflammation, curcumin, the main ingredient in turmeric, has shown promise in

the prevention and management of neurological disorders, including multiple sclerosis (Benameur et al., 2021). We carried out this study to investigate the sub-acute pancreatic and immunotoxicity of sodium fluoride and the probable restorative effect of curcumin on the pancreatic and immunotoxicity of sodium fluoride.

### **BIOCHEMICAL RESULTS:**

#### **As regards fasting blood insulin and glucose levels:**

When comparing the groups which received NaF to the control, there was a substantial statistical increase in fasting blood glucose associated with substantial statistical drop of fasting serum insulin. It is suggested that a few of the following elements may be involved in the process by which NaF causes hyperglycemia such as: Increased glycogenolysis brought on by an increase in cAMP: Increased release of adrenaline and a decrease in insulin secretion, which is expected to enhance the rates of gluconeogenesis and glycogenolysis while decreasing the rates of both (Tosur et al., 2019). By altering the intracellular signaling system linked to insulin secretion, fluoride has been shown to have an impact on insulin production (Rogalska et al., 2017). High fluoride levels have also been linked to beta-cell malfunction. Additionally, fluoride reduces the pancreatic tissue's response to glucose stimulation. (Zwierello et al., 2023). Numerous investigations demonstrated that a decrease in insulin secretion may be the cause of NaF-induced hyperglycemia. The hormone insulin, which is secreted by pancreatic  $\beta$ -cells, controls blood sugar levels. It has been shown that giving rats and humans F reduces insulin synthesis and lowers the hormones' plasma levels (Dilworth et al., 2021). Be in harmony with the present study Hameed et al. (2020), who demonstrated that providing oral sodium fluoride (100 ppm) to male albino rats in their water for 30 days caused the animals' blood glucose levels to rise noticeably above those of the control group. Similar results were published by Jaffer et al. (2022), who investigated how oral sodium fluoride administration of water containing 100 ppm NaF for 30 days affected the glycemic

index in rats. The results showed that while the serum insulin level dramatically dropped, the blood glucose level substantially rose. In contrast, Nunes et al. (2016) examined the impact of oral sodium fluoride (NaF) (50 mg/L) within drinking water for a period of 42 days on insulin signaling and insulin sensitivity in female Wister rats who had undergone ovariectomies. They reported that whereas blood glucose levels were similar in all groups, the OVX-F group had higher plasma insulin concentrations. On contrary to the present study, Rogalska et al. (2017), they looked at how rat peripheral tissues and the brain absorbed and transported [3H] glucose in response to postnatal fluoride exposure. Sodium fluoride (NaF) was administered to adult Wistar rats' drinking water for four weeks at a dosage of either 10 or 50 parts per million. According to the authors' hypothesis, there was a substantial difference in plasma insulin levels between the control and fluoride (50 ppm) groups, which grew as fluoride content in drinking water increased, even though there was no visible difference between the experimental groups. Because of the cells' lowered capacity to absorb glucose due to insulin resistance, Insulin levels have grown because the pancreas has to produce more of it to keep blood glucose levels within the normal level. Insulin resistance was measured by the HOMA-IR index, and the fluoride-treated rats had a greater score. This indicates that the treatment of NaF induced the development of insulin resistance (Nunes et al., 2016). When comparing those treated with curcumin against those treated just with NaF, The mean plasma glucose level was significantly lower statistically, whereas the mean insulin value was significantly higher statistically. It makes sense given that curcumin has anti-diabetic qualities. Theracurmin, a formulation that increases the bioavailability of curcumin when given orally to rats, has been shown to reduce glucose intolerance and raise plasma GLP-1 levels, which in turn triggers the release of insulin and subsequently lowers glucose (Kato et al., 2017). Additionally, glucose transporters (GLUT 1 and 3) expression is upregulated by curcumin, which lowers insulin resistance, and stimulating the IRS/PI3K/Akt pathway (Rivera-Mancía et al.,



2016). Additionally, curcumin has been shown to prevent high glucose-induced apoptosis in cultured neonatal rat cardiomyocytes via phosphorylating Glycogen Synthase Kinase 3 $\beta$  (GSK-3 $\beta$ ) and Akt (Yu et al., 2016). Curcumin was found to have comparable effects in vivo impact in rats with type 2 diabetes, including improvements in heart conditions such as fibrosis, oxidative stress, inflammation, and apoptosis, along with a decrease in blood sugar levels and cardiac dysfunction. The activation of Akt and GSK-3 $\beta$  by curcumin led to all of these results (Ren et al., 2020).

#### As regards the blood levels of immunoglobulins (IgG and IgM):

The current study's findings showed that all NaF-treated groups had statistically significant lower IgG and IgM levels than control groups. This is explained by Guo et al. (2017), who stated the fact that high fluoride intake causes downregulation of the B lymphocytes which are the major source of IgA, IgM, and IgG. Be in harmony with the present study Al-Harbi et al.

(2014), investigated how sodium fluoride affected young mice's immune systems. Following the intraperitoneal injection of 10.3 mg/kg b.w.t. NaF once a day for 30 days in a row. The results revealed that the level of both IgG and IgM showed marked decrease after the 4<sup>th</sup> week of treatment with NaF. In contrast to Guo et al. (2017), who looked into how mice's blood cellular and humoral immunity were affected by oral delivery of intragastric doses of 12, 24, and 48 mg/kg NaF for 21 days. The study's findings showed that there was no discernible difference in IgM and IgG between the 12 mg/kg group and the control group. Similar results were documented by Guo et al. (2017), who examined how oral sodium fluoride treatment (12, 24, and 48 mg/kg NaF) affected mice's blood cells and humoral immunity over the course of 21 days. As compared to the control group, the study's results demonstrated a significant decrease in both IgM and IgG in the 24 and 48 mg/kg groups. When curcumin was administered to groups IV and VI, the mean value of blood IgG and IgM levels increased statistically significantly when compared to NaF treated groups. Being in harmony with the present study Al-Harbi et al. (2014), who

investigated how curcumin can lessen sodium fluoride's immunotoxic effects on young mice. They proposed that during four weeks the amount of IgG and IgM in male mice with 40 mg/kg of intraperitoneal (I.P.) curcumin along with 10.3 mg/kg of NaF, would rise statistically significantly in comparison to the groups that received NaF treatment. The immunomodulatory qualities of curcumin, which can alter T and B cell activation, are how the authors explained this (Allegra et al., 2023). By boosting the production of Ab, curcumin treatment enhances humoral immunity. A surge in T follicular helper (Tfh) cells in the draining lymph nodes is most likely the mechanism causing this impact. In addition to the high affinity synthesis of IgG1 and IgG2b, curcumin also improves Abs function (Kim et al., 2019).

#### As regards oxidative stress biomarkers (MDA and TAC):

Entire antioxidant capacity is a metric that represents the whole activity of all the antioxidants found in plasma and body fluids, rather than just the simple sum of the measured antioxidants (Silvestrini et al., 2023). The primary metabolite of arachidonic acid, MDA, is a trustworthy biomarker for lipid peroxidation, which is indicative of oxidative stress being present (Cordiano et al., 2023). When comparing the mean value of MDA level to the mean value of TAC, the groups treated with NaF showed a statistically significant rise in the former while the mean value of TAC fell. The current outcome was ascribed to sodium fluoride's capacity to generate reactive oxygen species (ROS) and free radicals, resulting in a redox imbalance, both of which can cause cytotoxicity (Al-safei and Al-Mashhadane, 2021). The principal harm inflicted by reactive oxygen species (ROS) is lipid peroxidation of polyunsaturated fatty acids in cellular membranes, which leads to the secondary generation of malondialdehyde (MDA) (Cordiano et al., 2023). This indicates that fluoride depletes glutathione, increases MDA, and inhibits the activity of multiple antioxidant enzymes. It reduces the ability of cells to scavenge reactive oxygen species (ROS) by inhibiting ATPase action. This leads to a loss of ATP (Maheshwari et al., 2021). Intracellular

acidification is the result of ATP depletion, which causes ATP to hydrolyze into ADP and AMP and release protons. Oxidative stress is brought on by the release of free radicals from damaged mitochondria. DNA deterioration, disruption of metabolism, ATP hydrolysis, suppression of protein synthesis, intracellular acidification, and a reduction in their capacity to scavenge ROS are the results of this. Thus, produce more ROS (**Johnston and Strobel, 2020**). This was in agreement with **Al-safei and Al-Mashhadane (2021)**, who observed a substantial statistical rise in the mean value of MDA level in toxic groups relative to control groups following oral treatment of sodium fluoride at a rate of 20 mg/kg/day for 30 days in rabbits. This coincides with **Awad and Fakher Eldeen (2021)**, who found that after giving rats an oral dose of NaF (10 mg/kg) once a day for two weeks. When comparing the treatment group with NaF to the control groups, the total antioxidant dropped. In groups treated with curcumin, there was a significant statistical drop in the mean value of MDA level and a significant statistical rise in the mean value of total antioxidant. This outcome was ascribed to curcumin's ability to prevent oxidative damage. Curcumin enhances the antioxidant defense system and biochemical marker enzymes to produce its protective effects. Curcumin was found to have a significant protective effect against degenerative diseases. This protective effect was attributed to its ability to scavenge free radicals, which neutralize free radical ROS in mitochondria and other cellular parts. This approach to control mitochondrial oxidative stress involves inducing detoxification enzymes (**AL-Harbi et al, 2014**). By keeping antioxidant enzymes like glutathione peroxidase, catalase, and superoxide dismutase active at high levels, curcumin reduces lipid peroxidation. By interacting with and modifying antioxidant enzyme activity in response to degenerative illnesses, curcumin has indirect antioxidant benefits (**Kumar et al., 2018**). This was in harmony with **AL-Harbi et al. (2014)**, who showed that mice treated intraperitoneally (I.P.) with NaF at a dose of 10.3 mg/Kg for 30 days in a row had lower MDA than mice treated with a combination of NaF at a dose of 10.3 mg/Kg and

co-administered 60 mg/Kg of curcumin extract. Consistent with the present findings, **Nabavi et al. (2012)** confirmed that rats administered intraperitoneal doses of 10 and 20 mg/kg body weight of curcumin for seven days, followed by a seven-day period of 600 ppm sodium fluoride in their drinking water, demonstrated a normalisation of the decreased MDA level in their erythrocytes relative to the group that was administered NaF at the aforementioned dose. In line with the current findings, **Kumar et al. (2018)** found that rats treated with sodium fluoride at the same prior dose through drinking water and (20 mg/kg bw) of curcumin through gavage demonstrated a decrease in the level of MDA compared to rats given NaF (20 mg/kg) daily for 60 days.

#### **HISTOPATHOLOGICAL RESULTS:**

Fluoride is a vital substance for the growth of our body systems, particularly the formation of our bones and teeth. However, excessive consumption of fluoride can lead to deposits in various body tissues, particularly the pancreas, which can lead to pancreatic inflammation and damage (**Balaha et al., 2021**). In the present study, minimal histopathological changes were recorded in group III in the form of few degenerated acini with multiple congested dilated blood vessels in between them. Islets of langerhans showed some cells with vacuolations and dilated congested blood vessels. However, significant histological alterations were noted in group V, including numerous degraded acini and congested dilated blood vessels. Many inflammatory cells invaded the septa of connective tissue around blood vessels. There were more vacuolated cells and dilated blood capillaries in the islets of Langerhans. Some degenerated acini were replaced by homogenous highly acidophilic material. When rats were given water containing 100 ppm of sodium fluoride orally for 30 days, **Jaffer et al. (2022)**, observed degeneration and necrosis in pancreatic acini and ilets of langerhans. Our findings were similar to **Zaghloul et al. (2019)**, who looked into how adult male albino rats' pancreatic changes brought on by fluoride were affected by oral NaF therapy administered once daily for 35 days at a level of 10 mg/kg. The authors noted

that the blood arteries were dilated and became congested. They noted many cytoplasmic vacuoles, loss of typical acinar architecture and blood cells extravasated into the interlobular connective tissue. The explanation for the

which leads to cytoplasmic components dilatation consequent to intracellular fluid and electrolyte redistribution (**Aggrawal and Thakur, 2020**). In the present study, the administration of curcumin with sodium fluoride in both groups IV and VI showed normal pancreatic acini and islets of Langerhans as regard cells and blood vessels). The pancreatic islet cells' incredibly limited manifestation of antioxidative enzymes has led to the suggestion that the organ may be more vulnerable to oxidative stress than other tissues and organs. Since oxidative stress can trigger stress-sensitive signaling pathways, it is clear that oxidative stress contributes significantly to insulin resistance and  $\beta$ -cell dysfunction (**Bhatti et al., 2022**). Studies have revealed that curcumin can prevent lipid peroxidation, scavenge reactive oxygen species, and protect DNA and other cellular macromolecules from environmental deterioration. It is well recognised for having several uses, including as a potent antioxidant, diabetic control, anti-inflammatory, and anti-cancer agent (**Abdu El-Hamid and Moustafa, 2013**). This can also be explained by curcumin's capacity to dramatically raise insulin levels, reduce blood sugar, and halt the degeneration of pancreatic beta cells. Furthermore, curcumin may stimulate  $\beta$ -cell activity, which would raise insulin secretion (**Metawea et al., 2023**).

### CONCLUSION

Sodium fluoride affects the pancreas and immune system functionally and histologically and its toxic effect increases with increasing the dose. Also, it has been found that concurrent administration of curcumin induces apparent improvement.

### RECOMMENDATION

It is advised to:

1-To limit the use of toothpaste and other NaF containing products, and keep them out of reach of children.

formation of vacuolar degeneration in the pancreas is that fluoride toxicity causes the cell membrane to lose its selective permeability,

2- Conduct additional research on NaF detrimental effects on the immune system and pancreatic function.

3-Use curcumin as a powerful antioxidant natural compounds.

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## الملخص العربي

### دراسة التأثير التحسيني للكرامين علي البنكرياس، المناعة و الجهد التأكسدي في ذكور الجرذان البيضاء المسممة بفلوريد الصوديوم

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#### الملخص

مصر هي واحدة من ضمن 21 دولة تعاني من التسمم المتوطن بالفلوريد حيث ان لدي ايونات الفلوريد القدرة علي اختراق أغشية الخلايا و التراكم في الانسجة الرخوة المختلفة. وعلي الرغم من اجراء العديد من الابحاث علي التسمم بالفلوريد الا ان هناك عدد قليل نسبياً من الدراسات حول تأثيره علي الجهاز الهضمي مثل البنكرياس كما ان العلاقة بين الفلوريد و المناعة مجال مثير للابحاث. و من المتعارف علي ان الكركمين له عدة تأثيرات منها : انه مضاد للالتهابات، مضاد للاكسدة، الي جانب تأثيره علي ارتفاع معدل المناعة. **الهدف من البحث:** دراسة السمية تحت الحادة لفلوريد الصوديوم علي البنكرياس و المناعة و الجهد التأكسدي و التأثير التحسيني المحتمل للكرامين. **منهج الدراسة:** تم تضمين ستين من ذكور جرذان الالبينو البالغة. تم تقسيمه بشكل عشوائي الي ست مجموعات متساوية (10 جرذان لكل منها ) حيث استمرت الدراسة لمدة 4 اسابيع، المجموعة الاولى: (مجموعة ضابطة سالبة) ، المجموعة الثانية: تلقت الكركمين بجرعة 15.75 مجم/كم/يوم، المجموعة الثالثة: تلقت فلوريد الصوديوم NaF بجرعة 10مجم/كم/يوم، المجموعة الرابعة: تلقت فلوريد الصوديوم NaF بجرعة 10مجم/كم/يوم بالإضافة لجرعة الكركمين السابق ذكرها، المجموعة الخامسة: تلقت فلوريد الصوديوم NaF بجرعة 25 مجم/كم/يوم، المجموعة السادسة: تلقت فلوريد الصوديوم NaF بجرعة 25مجم/كم/يوم بالإضافة لجرعة الكركمين السابق ذكرها. تم قياس مستويات الانسولين و الجلوكوز و IgG و IgM و MDA و TAO . و تم اخذ انسجة البنكرياس لفحصه بالمكروسكوب الضوئي هستولوجيا. **النتائج:** تشير نتائج الدراسة الي نقص ذو دلالة احصائية في مستوي كلا من الانسولين، IgG، IgM، TAO مع وجود ارتفاع ذو دلالة احصائية في مستوي كلا من الجلوكوز و MDA في كلا من المجموعتين الثالثة و الخامسة. كما تبين وجود ارتفاع ذو دلالة احصائية في مستوي كلا من الانسولين، IgG، IgM، TAO مع وجود نقص ذو دلالة احصائية في مستوي كلا من الجلوكوز و MDA في كلا من المجموعتين الرابعة و السادسة . وجدت تغيرات باثولوجية بانسجة البنكرياس في المجموعة التي تلقت فلوريد الصوديوم مع وجود تحسن ملحوظ مع اعطاء الكركمين. **الاستنتاج:** من النتائج المتحصل عليها، وجد ان فلوريد الصوديوم يؤثر علي البنكرياس و الجاز المناعي و الجهد التأكسدي ان اضافة الكركمين تؤدي الي تحسن ملحوظ.

**الكلمات المفتاحية:** فلوريد الصوديوم، كركمين، بكرياس، مناعة، الجهد التأكسدي .