Original Article

DNA Deterioration and Biochemical Changes Induced by Sodium Fluoride on Rat Kidney and the Potential Effect of Resveratrol

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ABSTRACT

Introduction: Fluorosis can be caused by long-term fluoride exposure. The kidney is one of the organs most commonly harmed by fluoride. In cytotoxic amounts, fluoride can cause oxidative changes, histopathological lesions and DNA deterioration. Resveratrol is a potent natural antioxidant minimizing oxidative damage induced by many toxins in the different tissues.

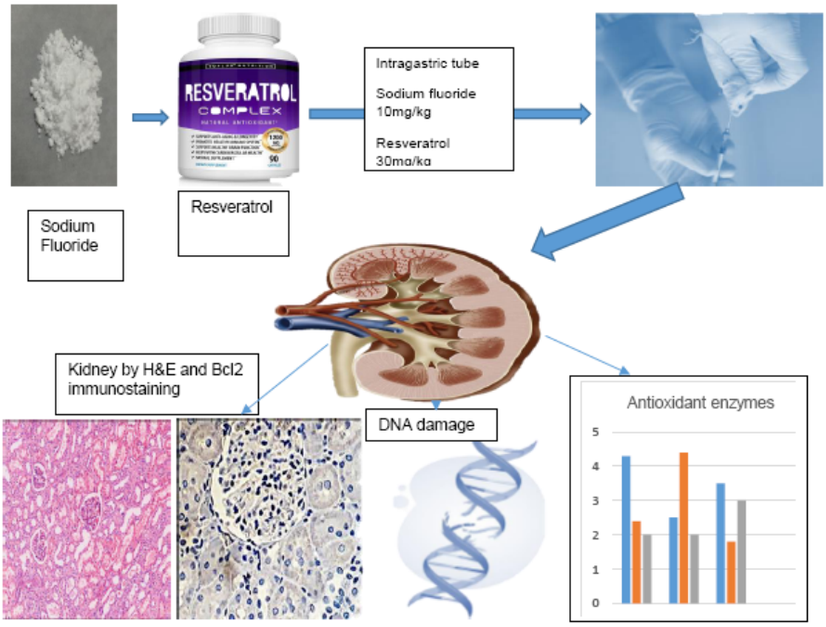
Aim of the Work: The goal of this research is to find out how much Sodium Fluoride (Na F) affects kidney cells, its oxidative enzymes and its DNA and how Resveratrol (RSV) can prevent and repair it.

Materials and Methods: 36 adult male albino rats were used in this research divided into 3 main groups: control group further subdivided into 2 subgroups, Na F treated received10 mg/kg b.w of Na F and the protected group (Na F with resveratrol) which received both 10 mg/kg b.w of Na F and 30 mg/kg b.w of resveratrol. The experiment lasted for 30 days. The toxicity of Na F was assessed histologically, biochemically, and by using Comet assay.

Results: In the Na F-treated group, histological investigation indicated abnormal cellular pattern in the form of cellular vacuolation, intertubular hemorrhage, and cellular infiltration, as well as increased Bowman's space thickness along with a lower Bcl-2 percentage. In the cellular homogenate of the Na F treated group kidneys, biochemical data revealed altered antioxidative enzymatic levels; MDA levels were increased significantly along with a decrease in SOD& GPX levels. In addition, there was increased DNA damage which was indicated by Comet assay. The majority of these effects, however, were improved when Resveratrol was administered concurrently.

Conclusion: Resveratrol can mimic the effects of sodium fluoride on the kidneys.

Graphical Abstract



DNA deterioration and biochemical changes induced by Sodium Fluoride on Rat kidney and the potential effect of Resveratrol

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INTRODUCTION

Fluoride is a trace element that is required by the human body. It has a wide natural distribution and is widely used in a variety of industries, including pharmaceutical preparations, agriculture, insecticides, and surfactants. Many countries have added it to their drinking water supplies because of its favorable benefits on tooth and bone mineralization. However, excessive fluoride consumption by human and experimental animals, whether accidental or suicidal, has been found toxic to a variety of body parts, including the reproductive organs and neurons. Fluorosis of the teeth and skeleton, infertility, and mental retardation are all possible side effects[1].

Drinking water, particularly soft, alkaline, and calcium-deficient waters, is a major source of fluoride poisoning[2]. Food, industrial exposure, drugs, cosmetics, and other sources of fluorine can all enter the body[3].

Fluoride is absorbed primarily by the intestine, then into plasma, where it is dispersed to various body tissues and fluids. Because the kidneys are the primary site of fluoride excretion, urinary excretion accounts for 50% to 80 % of fluoride excretion[4].

Chronic Fluoride poisoning is associated with increased oxidative stress in the liver, brain, and heart in both humans and animal models[5]. The antioxidant enzymes are inhibited by fluoride, which promotes the buildup of reactive oxygen species (ROS). Fluorine can also cause genotoxicity, cytotoxicity, immunotoxicity, and apoptosis in several human organs, according to previous research[6].

Resveratrol (trans-3,5,4-trihydroxystilbene) is a phytoalexin found in grapes, plums, cranberries, and peanuts[7]. It is a potent antioxidant because it reduces oxidative damage caused by a variety of toxicants in animals. It possesses anti-inflammatory, antioxidant, and chemo preventive effect. Treatment with resveratrol has been shown to be effective in the treatment of aging symptoms, cardiovascular disease, diabetes, and cancer[8].

The present study investigated effect of subchronic fluoride exposure on rat kidney as well as the effect of concomitant use of resveratrol.

MATERIALS & METHODS

Chemicals

Sodium monofloride (Na F) as anhydrous powder 99.99% trace metal basis and Resveratrol 3,4′,5-Trihydroxy-trans-stilbene, 5-[(1E)-2-(4-Hydroxyphenyl) ethenyl]-1,3-benzenediol. 99% purity. Both were obtained from Sigma-Aldrish chemicals company, Germany.

Animals

In this experiment, 36 adult male albino rats weighing 150-250 g were employed. All were collected from the Animal House of Zagazig University's Faculty of Medicine, where they were adapted for 14 days. They were maintained in separate well ventilated cages, under standard settings, with free access to standard meal and water ad libitum, prior to the start of the experiment to achieve optimal physical conditions. The Ethics Committee of Zagazig University, Institutional Animal Care and Use Committee, granted ethical approval for the experimental method (ZU-IACUC/3/F/185/2021). Following the acclimatization phase, the rats were separated into three groups twelve in each group.

Methods

Experimental design

Rats were separated into three groups, twelve in each group. Group I is the control group, this group was divided into 2 main subgroups each of which contains 6 rats; negative control(Ia) in which rats were given no treatment all through the experiment and positive control (Ib) in which rats were given Resveratrol at a daily dose of 30 mg/kg b.w through intragastric tube(IGT) for 30 days[10]. Group II is the experimental group (Na F treated group), rats were administered with 10mg/kg b.w of Na F dissolved in 2.5 ml distilled water through IGT once daily for 30 days[9]. The rats in Group III (Resveratrol and Na F group) were given both 10 mg/kg b.w of Na F and 30 mg/kg b.w of resveratrol once daily for 30 days by IGT[10]. The rats were anesthetized with 25% chloral hydrate at the end of the experiment, and a laparotomy was carried out to extract the kidneys. Following that, renal samples were either placed with phosphate - buffered saline (20 % homogenates) or fixed in 4% formalin for histological analysis.

Biochemical analysis

lipid peroxidation estimation& oxidative enzyme essay

To isolate the kidneys homogenates, the supernatants were centrifuged at 1000 g for 20 minutes at 4 c. The supernatant was investigated for MDA, SOD, & GPX

- Lipid peroxidation estimated by measuring malondialdehyde (MDA): It was measured colorimetrically in renal homogenate utilizing a commercially available kit (Biodiagnostic, Cairo, Egypt) in accordance with[11]
- Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) were assessed in renal homogenate in accordance with[12] utilizing a commercially available kit (Biodiagnostic, Cairo, Egypt).

Comet assay

According to[13] we employed a modified alkaline comet assay methodology. On thoroughly frosted slides, six microliters of renal homogenate were deposited on 0.5 % weak agarose, placed between a 0.6 % normal-melting agarose layer and a 0.5 % weak agarose upper layer. During the polymerization of each gel layer, the slides were held on ice. Then they were submerged in a lysis solution at 40°C after the 0.6 % agarose layer solidified. The slides were inserted in electrophoresis buffer to make

it possible for DNA to unwipe. At 300 mA and 1 V/cm, electrophoresis was carried out for 10 minutes. The slides were neutralized with pH 7.5 Tris-HCl solution before being stained for 10 minutes with 20 g/mL ethidium-bromide. The epifluorescence microscope Leitz Orthoplan (Wetzlar, Germany) was used to examine each slide. To measure tail length and intensity, the comet assay II automatic digital analysis equipment was used to assess 100 cells on each slide. The analysis was done at Animal Reproduction Research Institute (ARRI), Cairo.

Light microscopic examination

Fixed formalin samples were processed in paraffin wax. sections of (5 µm) thickness were collected and prepared for the following stains:

1. Hematoxylin and Eosin stain (H&E) according to[14]: After mounting on glass slides, 5 µm thick sections were deparaffinized in xylene and stained with hematoxylin and eosin (H&E). Light microscopy (The Leica DM500 with Leica ICC50 W Camera Module) was used to examine stained slides at the Image Analysis Unit of the Anatomy and Embryology Department, Faculty of Medicine, Zagazig University.
2. Periodic acid Schiff (PAS)stain: to illustrate carbohydrates[14]
3. Masson trichrome stain: to study connective tissue proliferation[14]
4. Immunostaining for Bcl-2 (B-cell lymphoma 2)[15].

The sections were mounted on charged slides, deparaffinized, and washed in phosphate-buffered saline. Heating unmasked the antigen sites, and 3 %H_2O_2 inactivated the endogenous peroxidase. The sections were blocked for 1 hour with 10% (w/v) normal goat serum before being treated overnight at 4°C with mouse monoclonal anti-Bcl-2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After that, all samples were treated for 30 minutes at 37° C with biotinylated secondary (Dako Cytomation, USA) antibodies (1:1000). The chromogen 3,3′-diaminobenzidine tetrachloride technique was used to visualize the specific protein immunoreactivity under 400x magnification using an Olympus camera (Nikon Eclipse E200-LED, Tokyo, Japan).

Morphometric analysis

At a magnification of 400, the Bowman's space thickness, and also the regions that have been positively labeled (brownish painted areas) of Bcl2, were all measured morphometrically. The data were quantified using "Image J" 1.49v/Java 1.6.0_244″, a free image processing program (National Institutes of Health, USA). Initially, the image analyzer was calibrated automatically to transform the image analyzer program's measurement units (pixels) into actual micrometer units. Ten non-overlapping fields from five serial sections from the slides of each specimen in each group were scored at random. The data was recorded, displayed as a mean ± standard deviation (SD), and statistically analyzed[16].

Statistical Analysis

The data were examined using one-way analysis of variance (ANOVA) and Tukey-Kramer multiple comparison test. The results were judged significant if $P<0.05$. Statistical Package of Social Sciences version 22 (SPSS) software was used for the analysis.

RESULTS

Biochemical results

Regarding Lipid Peroxidation, In the fluoride alone treated group, the MDA levels in kidney tissue was significantly higher than those of the control group ($p < 0.05$). The presence of resveratrol reduced the elevation of MDA levels. There was a statistically significant change in MDA levels in kidney tissue observed in all groups (Table 1, Figure 1)

The activity of SOD& GPX in the kidney was used to determine the antioxidative state. The findings revealed that there were considerable disparities in SOD and GPX activity. There was a significant statistical difference in SOD and GPX between the three studied groups. In addition, SOD and GPX activities were significantly higher in the kidneys of rats co-treated with Resveratrol and fluoride than in fluoride-only-treated animals ($p < 0.05$) (Table 1, Figure 1)

Comet test results

Genomic DNA damage has been presented in (Figure 2). Exposure to Na F caused a significant increase in DNA breaks resulting in increased migration of DNA from nucleus to tail of comet with some improvement with resveratrol administration. There was a significant difference between the 3 groups (Table 2, Figures 2,3)

Histological Results

H&E results

H&E examination of control (positive, negative) rat kidney revealed a normal glomerular pattern surrounded by normal Bowman's capsule and space in the renal cortex as well as intact tubules lined by cells containing vesicular nuclei (Figures 4 A,B). In Na F treated group, there was atrophied shrunken glomeruli with widened Bowman's space. The tubules of the kidneys were also altered in various ways, including dilatation of their lumens, thinning of their lining cells which may contain darkly stained pyknotic nuclei and cytoplasmic vacuolation. There was peritubular hemorrhage in the interstitium. Also there was hyaline casts seen in some tubules with extensive fibrosis around congested blood vessels and cellular infiltration (Figures 4 C,D). Resveratrol treatment improved the histological profile of the kidneys to some extent. Normal glomerulus and Bowman's space were seen in rats given Na F and resveratrol. The majority of tubules retained their typical appearance with vesicular nuclei, although

others were still dilated with shrunken cellular lining. Also, peritubular hemorrhage was seen at some areas (Figure 4E).

PAS results

PAS staining of the control group revealed that the brush boundaries on the apical side of the tubular lining cells, the thin parietal layer of Bowman's capsule, and the thin basal lamina of the kidney tubules were PAS positive. In Na F treated group: The tubular epithelial cells showed disruption with absence of brush boundaries at some regions, there was thickening in parietal layer of Bowman's capsule of the glomeruli and the tubule basal membrane. In Resveratrol protected group: there was some preservation in the tubular brush boundary as well as the tubular basement membrane and glomerular basement membranes (Figure 5).

Masson trichrome results

Masson's trichrome staining of renal sections showed that, compared with control groups, interstitial fibrosis was much larger in Na F treated rats, this fibrosis was seen in between the tubules and around glomeruli. while it was scarcely found in Resveratrol protected groups (Figure 6)

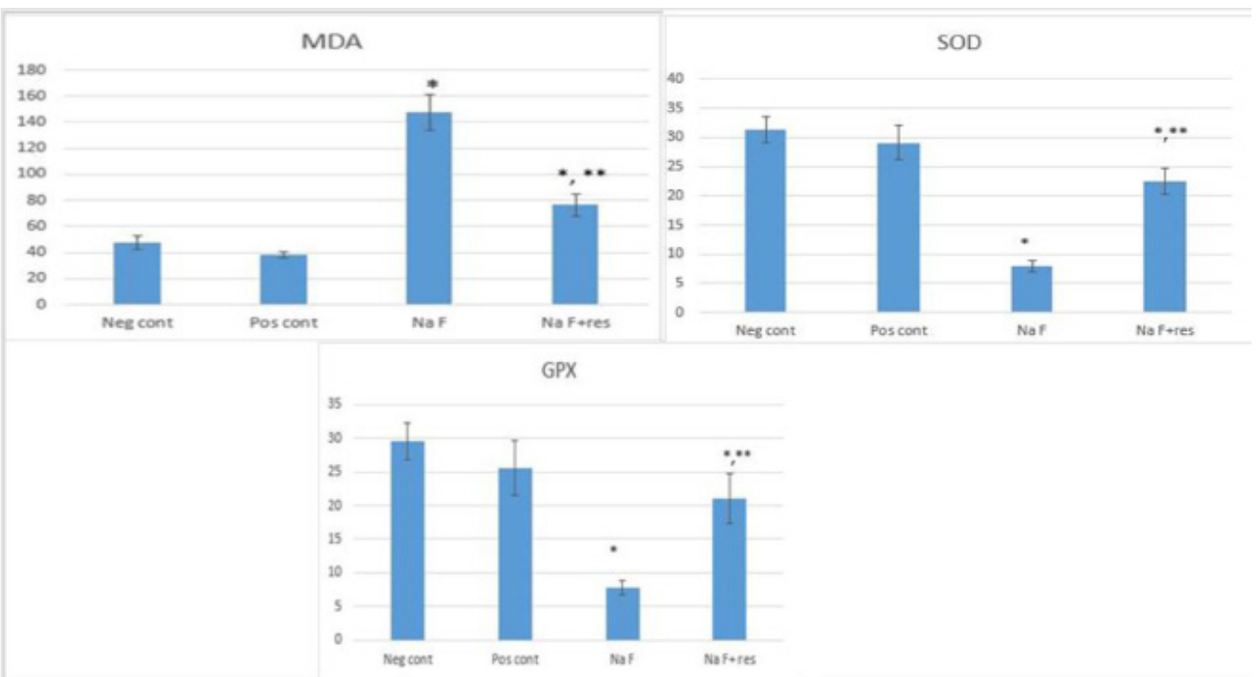
Bcl-2 results

Bcl-2 immunohistochemical staining of renal cortex sections revealed positive cytoplasmic immunoreactivity which was weak in Na F treated group, moderate in the control group, and strong in Na F and resveratrol group (Figure 7).

Morphometric results

Regarding diameter of Bowman's space, H&E sections statistical analysis showed significant($P<0.05$) difference between control and Na F treated group. Also there was a significant difference between Na F treated alone and the group treated with both Na F and Resveratrol at the same time. There with no statistical significance between control and resveratrol treated groups as shown in (Figure 4F, Table 3)

Regarding Bcl-2 area percentage, there was significant increase in Na F and Resveratrol protective group when compared with control and Na F only group (Figure 8).



* significant against control **significant against Na F

Fig. 1: bar charts for MDA, GPX and SOD tissue levels in all groups

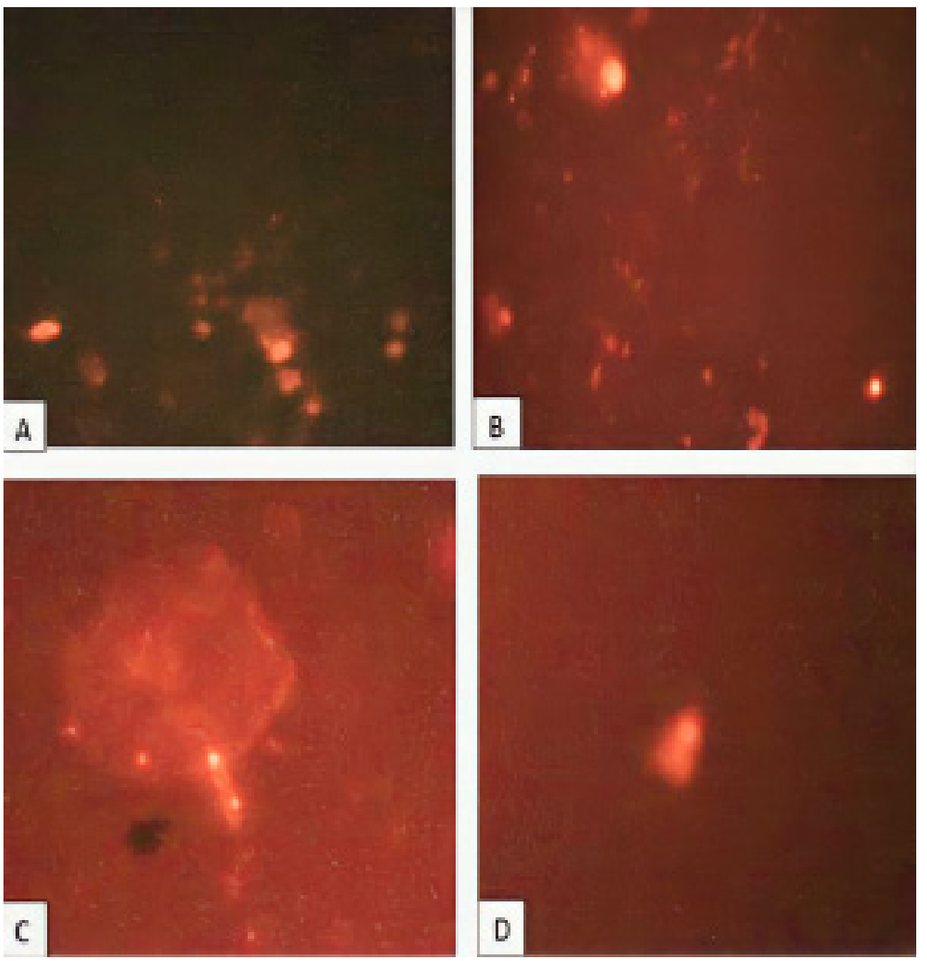
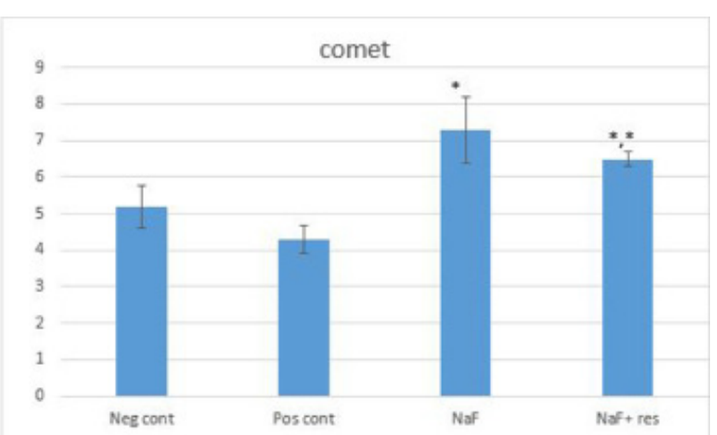


Fig. 2: A photomicrograph of comet formation of rat kidney at 400x magnifications in control (A&B), treated with Na F(C) and protected (Na F+ resveratrol) (D).



* significant against control **significant against Na F

Fig. 3: A bar chart for results of Comet's test (tail DNA percent means levels) in all groups.

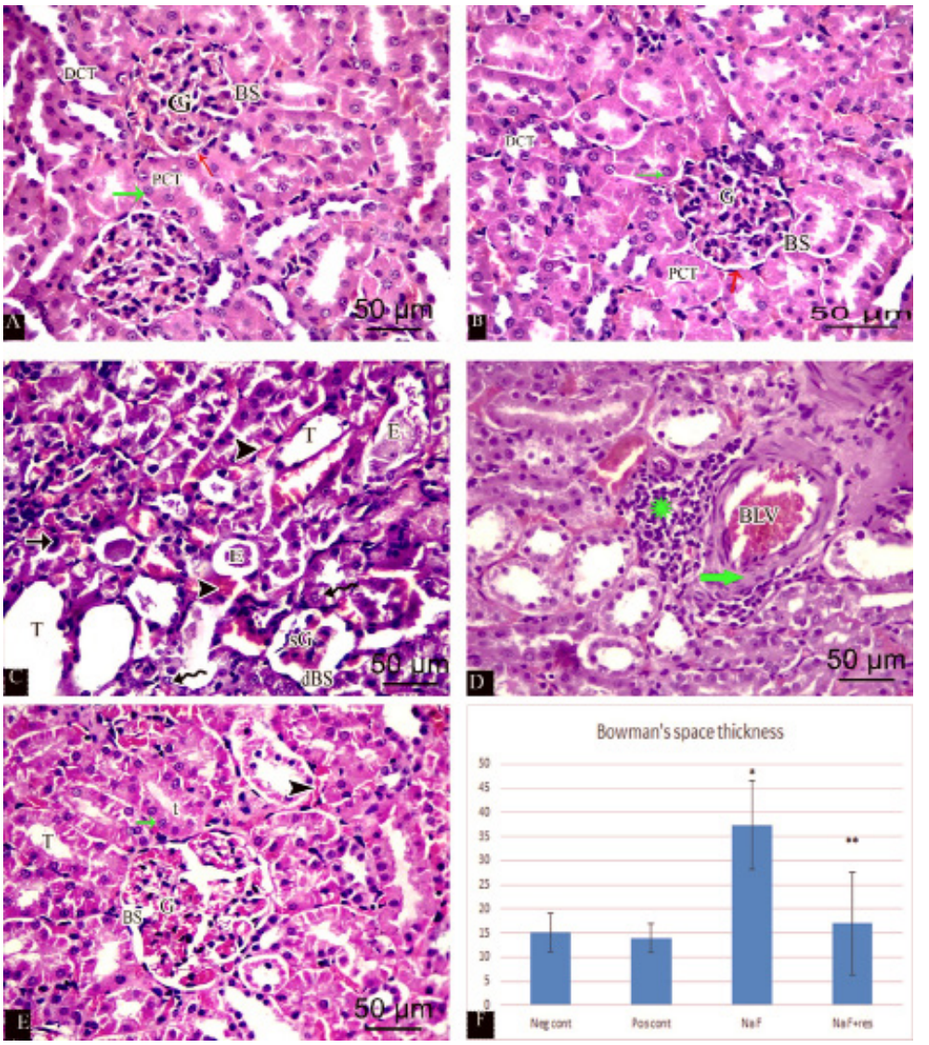


Fig. 4: A photomicrograph of H&E stained kidney tissue sections at 400x magnifications of different groups showing normal glomeruli (G) surrounded by Bowman's capsule (red arrow) and space (BS). Intact proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) lined by cells containing vesicular nuclei (green thin arrows) in control group (A: control negative &B control positive). Na F treated group (C, D) showing shrunken glomeruli (sG) with dilated Bowman's space (dBS). Epithelial cells lining the renal tubules appear thin containing darkly stained pyknotic nuclei (black arrow) and vacuolated cytoplasm (zigzag arrow). The lumena of the renal tubules are dilated (T) and contains eosinophilic material (E). peritubular haemorrhage (arrow heads) is also seen in between the tubules. Also blood vessels are congested and filled with haemorrhage (BLV) and surrounded by excessive fibrous tissue (green thick arrow) with extensive cellular infiltration (green star). The resveratrol with Na F protected group (E) shows some restoration of the normal kidney structure with normal glomerulus (G) surrounded with normal Bowman's space (BS), most of the tubules retain their normal appearance (t) with lining vesicular nuclei (green arrow). However, some tubules still affected dilated and thinned (T) with some peritubular haemorrhage (arrow heads). H&E* 400
(F): A bar chart for Bowman's pace thickness in all groups: in Na F treated group: The Bowman's pace thickness is significantly increased in relation to both control groups and Resveratrol protected group.

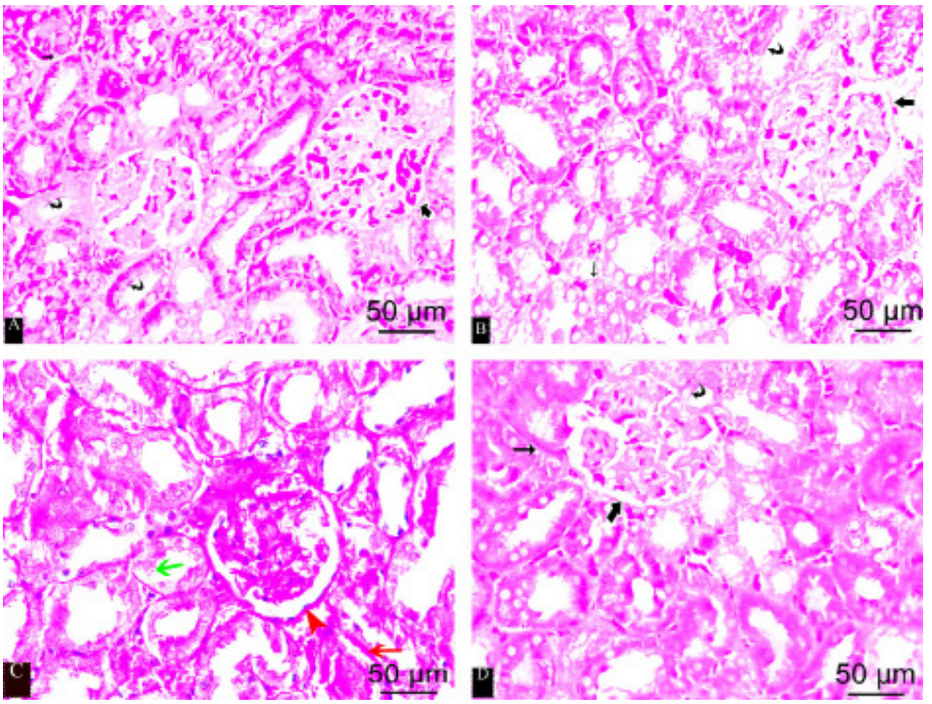


Fig. 5: A photomicrograph of Periodic Acid chief stained kidney tissue sections at 400x magnifications of different groups showing well-formed tubular basement membranes (thin arrow), Basement membranes of glomeruli appear as thin regular PAS +ve (thick arrows). Also, there is well developed brush borders of convoluted tubule (curved arrows) in both negative control subgroup (A) and positive control subgroup (B). in the Na F treated group (C), There is a strong positive PAS reaction of thicker basement membrane of both glomeruli (red arrow head) and renal tubules (green thin arrow) as well as discontinuous PAS reaction at tubular cell brush boundaries (red thin arrows). Na F with Resveratrol showing; PAS positive reaction at the thin Bowman's capsule (thick arrows) and tubular basement membrane (thin arrows) and continuous brush border of tubules (curved arrows). PAS* 400

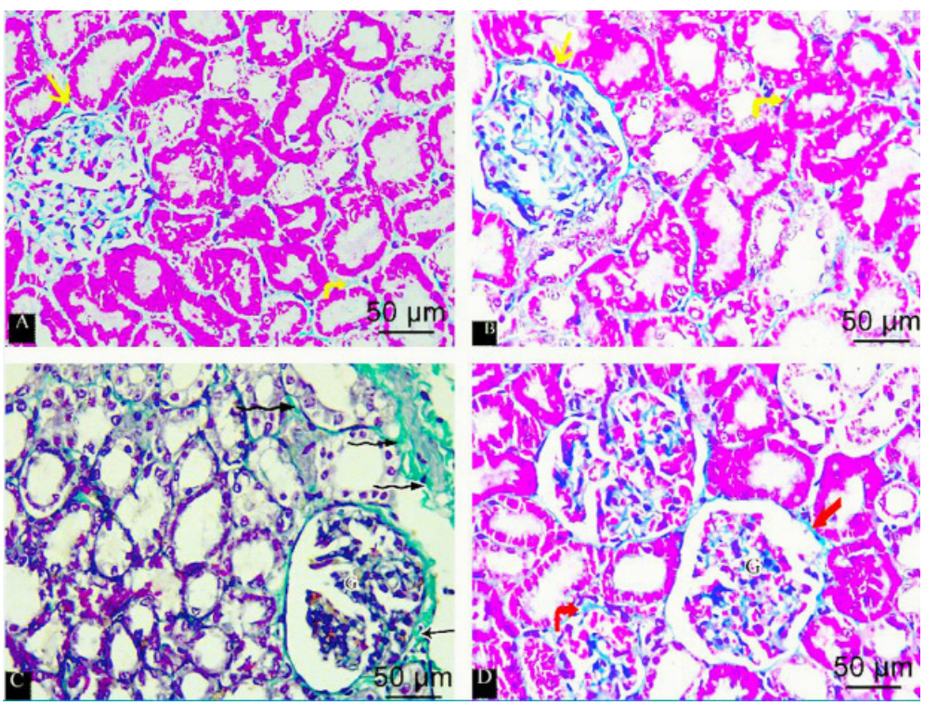


Fig. 6. A photomicrograph of Masson's trichrome stained kidney tissue sections at 400x magnifications of different groups showing no abnormal collagen deposits around glomeruli (yellow arrow) and between tubules (yellow right angled arrow) in both negative control subgroup (A) and positive control subgroup(B). Na F treated group(C), showing marked amounts of collagen deposition around renal glomeruli (black arrow) and around tubules (black wavy arrow). Na F with Resveratrol (D) showing minimal collagen deposits around glomeruli (red arrow) and between tubules (red right angle arrow). Masson's trichrome*400

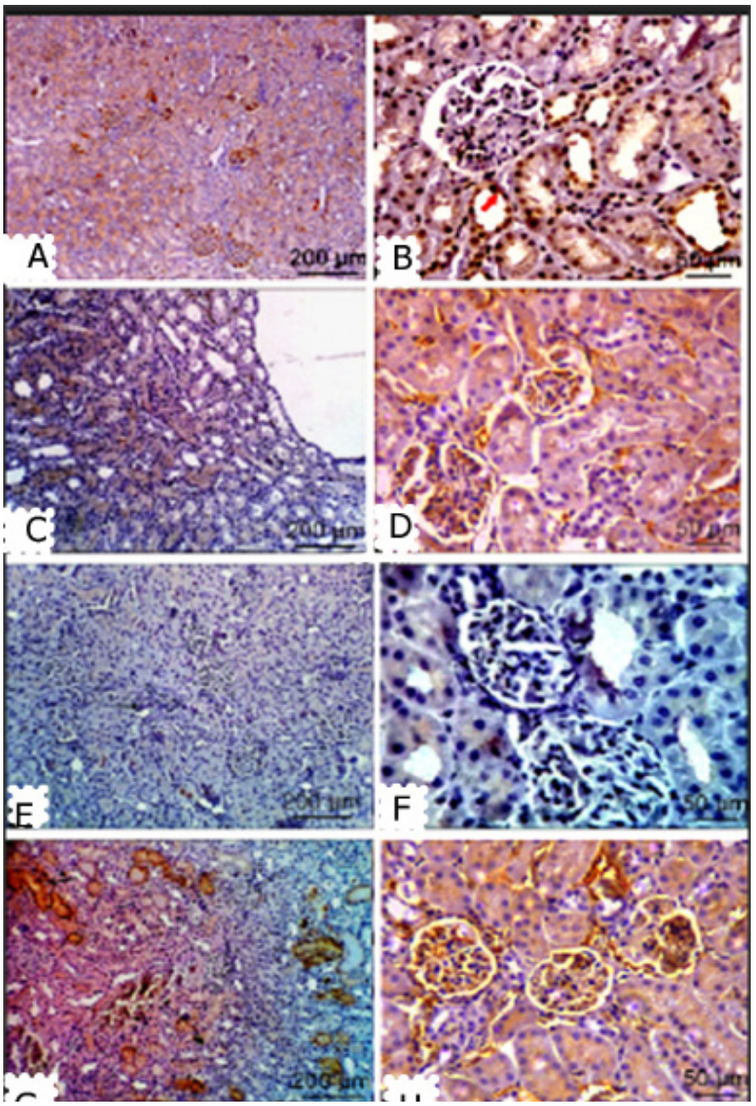
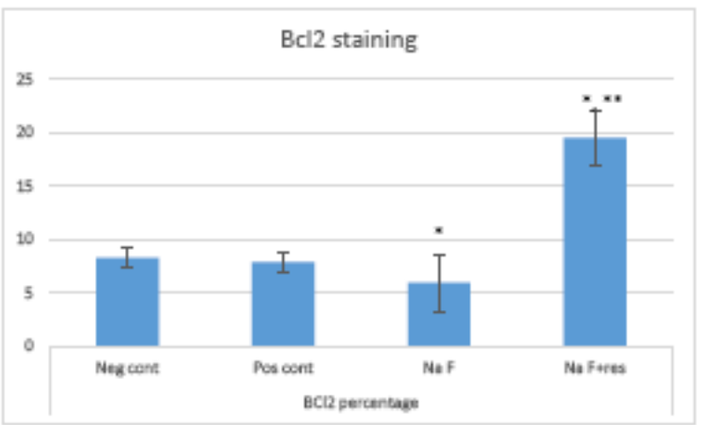


Fig.7: A photomicrograph of BcL2 immunohistochemical staining of renal cortex sections at 100, 400x magnifications revealed positive cytoplasmic immunoreactivity in both negative control subgroup (A, B) and positive control subgroup (C, D) weak in the Na F treated group (E, F), and strong in Na F and resveratrol group (G, H). BcL2*100, 400



* significant against control **significant against Na F

Fig 8: A bar charts for BCL2 staining area in all groups

Table 1: Biochemical and homogenate outcomes among several research groups

	Control group (n 12)		Na F (n 12)	Na F+ resveratrol group (n 12)	*P value*
	Negative control(n 6)	positive control(n 6)			
MDA (mg/dl)	47.8±5.1	37.9±2.5	147.5^{a} ±13.65	76.55^{ab} ±8.4	0.0000*
GPX (ng/ml)	29.56±2.79	25.6±4	7.82^{a} ±1.04	21.03^{ab} ±3. 64	0.0000*
SOD(u/ml)	31.27±2.2	29.1±3	7.9^{a} ±0.98	22.5^{ab} ±2.27	0.0000*

One-way ANOVA test was used to assess the findings. The findings are mean± standard deviation (n = 12). The data for control animals is used as a baseline
a: significant from the control group b: significant from Na F group *significant one-way ANOVA test

Table 2: comet assay tail length outcomes among several research groups

Comet assay	Control group (n 12)		Na F (n 12)	Na F+ resveratrol group (n 12)	*P value*
	Negative control(n 6)	positive control(n 6)			
	5.2±0.59	4.3±0.39	7.3^{a} ±0.90	6.5^{ab} ±0.22	0.0000*

One-way ANOVA test was used to assess the findings. The findings are mean± standard deviation (n = 12). The data for control animals is used as a baseline
a: significant from the control group b: significant from Na F group *significant one-way ANOVA test

Table 3: Thickness of Bowman's space & Bcl-2 percentage area outcomes among several research groups

	Control group (n 12)		Na F (n 12)	Na F+ resveratrol group (n 12)	*P value*
	Negative control(n 6)	positive control(n 6)			
Thickness of Bowman's space	15.15 ± 3.9	14 ± 3	37.4^{a} ± 9.2	16.96^{ab} ± 10.7	0.0000*
Bcl-2 percentage	8.33 ± 0.97	7.9 ± 0.9	5.966^{a} ± 2.7	19.5^{ab} ± 2.15	0.0000*

One-way ANOVA test was used to assess the findings. The findings are mean± standard deviation (n = 12). The data for control animals is used as a baseline
a: significant from the control group b: significant from Na F group *significant one-way ANOVA test

DISCUSSION

Fluoride is a naturally occurring chemical that is frequently employed in industry and is particularly abundant in nature. It can be found in nearly all environmental matrices[17]. It plays an important function in preventive dentistry due to its high cariostatic capacity. It's found in a variety of toothpastes and mouthwashes, as well as treated nutrition. The widespread usage of fluoride products resulted in a slew of health issues[18].

Fluoride has been demonstrated to be toxic to numerous organs, including kidneys, reproductive organs, and neurons, and can cause dental and skeletal fluorosis, fertility problems, and developmental delay, among several other problems[19,17].

According to Nabavi and his coauthors, the kidneys are the key sectors implicated in Na F elimination and storage, hence Na F poisoning caused severe damage to the kidney and its function[20].

H&E stained sections of kidney in this experiment showed pathological changes in renal cortex of Na F treated rats in the form atrophy of corpuscles. This was in agreement with[21] who added Na F in different concentration to gunia pigs'food and found this pathology mostly with all concentrations.

This study's findings of cellular vacuolation and interstitial hemorrhage between renal tubules obtained from Na F treated rats were consistent with those of[18]who treated adult rats with 10 mg Na F for 4 weeks and found diltation of tubular lumens, particularly in the proximal convoluted tubules. This was explained by[22] who claimed that Fluoride affects mitochondrial activity causing inhibition of kidney function in addition, it prevents cellular respiration through generation of free radicals resulting in renal failure.

Basha and other co-investigators Subjected mice to fluoride for 15 days and 30 days, and found that the 15-day exposure resulted in glomerulus necrosis, degenerative changes in Bowman's capsule, tubular region and abnormalities in the glomerulus. Furthermore, as compared to the other group 30 days later, these pathological lesions were more severe, with the formation of large vacuoles and widening of the lumen of distal convoluted tubules[23].

In the current study, cells of the Fluoride-treated group displayed cytoplasmic vacuolations as reported by[24] who found the same when added Na F to the drinking water of albino rats and reported cellular vacuolation with tubular dilatation. Also, this was claimed by[25] who looked into the impact of Na F on the rat renal tissue of mothers and their first generation and discovered that Na F caused many ultrastructural changes in both studied ages, including larger mitochondria, vacuole formation, and apoptosis, all of which was attributed to ROS production or inhibition of antioxidant enzyme activity with increased malondialdehyde generation in the kidney.

In this experiment, extensive fibrosis was seen around congested blood vessels by H&E and by Masson trichrome and also around tubules and glomeruli in rats treated with Na F. This was also found by[26] on studying effect of hard water with extra concentration of different metals such as Fluoride, Cadmium and Aluminum and other metals. In the former study, extensive kidney fibrosis and sclerosis was observed in mice treated with Fluoride hardened water as with other types of metals.

Several investigations have shown that fluoride toxicity is linked to increased production of reactive oxygen species (ROS) and a loss in antioxidant defenses[27]. This was in line with the biochemical findings of the current investigation. MDA levels were substantially greater in the Na F treated kidneys than in the control group. In addition, the levels of SOD and GPx in the Na F-exposed group were considerably lower than those of the control group. This was consistent with[28-31]. Results from this study showed that MDA, SOD, and GPx levels improved marginally when rats were given resveratrol and Na F at the same time[32]. discovered the same issue when treating rat with glycerol with resultant reduced renal activities of GSH, SOD and reported that, these changes where improved with the pretreatment with Resveratrol.

Another mechanism by which Na F affects kidney is illustrated by[18] who found upregulation of Toll-like receptors (TLRs) in kidneys of Na F treated rats. TLRs are important components of the innate immune system because they activate leukocytes and induce proinflammatory signaling cascades[33]. Tubular epithelia, mesangial cells, and invasive macrophages all exhibit TLRs in the kidney. It has been proven that TLRs are activated in these cells during renal fibrosis. TLRs are engaged in the majority of renal inflammation. TLR1,2,3,4, and 6 are expressed by renal tubular cells, implying that these sensors are implicated in the activation of host defense in tubule interstitial injury[34]. The first investigators stated that N-acetylcysteine (NAC) and/or thymoquinone (THQ) administration alone or in combination downregulated TLR-4 protein expression.

In the current study, some tubular lumens were filled with hyaline casts. this was described in an experiment performed by[30]. The previous authors detected gradually changing renal histopathological lesions and disturbed function parameters with change in Na F dose and exposure time in mice treated orally with 0, 12, 24 and 48 mg Na f / kg body weight for 42 days.

In this study, we found some alteration in the tubular brush border in Na F treated group, this was also evidenced by[21] who found damage of the apical brush boundary membrane of the proximal tubular epithelium in fluoride-treated young pigs by transmission electron microscopy. We also described distortion in the tubular basement membrane in the form of thickening or degeneration as was detected by[35] in an experiment performed on rats for 6 weeks. In an experiment performed by[36], it was proved that Na F in hard water injured tubular brush border at concentration300 mg/kg and caused thickening of basement membrane at concentration600mg/kg for 40 days. However, these alterations were improved by Resveratrol treatment as described by[37] in an experiment performed on rats exposed to different doses of trifluralin.

Resveratrol (RSV) has been shown to protect against a variety of oxidative stressors and inflammation. It also contains anti - oxidative, antimutagenic, anti-inflammatory, estrogenic, antiplatelet, and anticancer properties and cardioprotective activities[38,39]. RSV exhibits antioxidative and free radical scavenging characteristics, as it reversed the effects of Na F on hydrogen peroxide levels and catalase activity. Resveratrol helped to restore Glutathione S transferase (GST) activity that had been disrupted by Na F[40]. In addition, it was stated that, Na F decreased total thiol levels which indicates oxidative stress, and resveratrol restored total thiol levels completely, indicating its antioxidative ability. Moreover, Resveratrol has the ability to suppress the Na F-mediated increase in nitric oxide (NO) concentration which indicates its anti-inflammatory properties[41].

The findings of dilated Bowman's capsule space in the Na F-treated kidney as compared to the control group were similar to those of[42], who observed dilatation of Bowman's capsule spaces in fluoride-treated Swiss albino mice with minimal improvement in withdrawal group. Also[31] found the same issue in rats treated with 15 ppm of Na F in their drinking water for 90 days.

ROS have been linked to apoptosis as potential modulators. Lipid peroxidation, protein and nuclear changes have all been linked to oxidative stress's capacity to induce apoptosis as a result of significant cellular damage. Fluoride-induced apoptosis was shown to be produced by oxidative stress in previous research, implying that oxidative stress plays a role in the apoptotic process. A member of the Bcl-2 family has been found to play a role in apoptosis regulation. Apoptosis is inhibited by Bcl-2[43].

They belong to the Bcl-2 protein family, which regulates the outer mitochondrial membrane integrity during apoptosis. Intrinsic transmembrane switch inhibition has been demonstrated for Bcl-2. Also, Bcl-2 may prevent apoptosis by sequestering the caspase precursor[44], restricting the release of Cyt c, or lowering mitochondrion malfunction, among other methods[45,46].

In terms of Bcl-2, There was a significant difference in area %for Bcl-2 across the three groups tested in this study. This was in agreement with[47] who exposed 240 ICR mice, aged 30 days to different concentrations of Na F for different periods and discovered that at 21 and 42 days of the trial, Bcl-2 levels were considerably lower in the various treatment groups.

In a study of the effect of Na F and Maize Purple Plant Pigment as an important antioxidant on rat liver and kidney,

the researchers discovered that Bcl-2 level was reduced in the hepatic and renal tissues of fluoride-treated rats, but that Maize Purple Plant Pigment can counteract this drop. The recent discovery supports the hypothesis that oxidative stress contributes to apoptosis[29].

Fluoride chemicals have been found to produce genetic changes in a variety of domains, promote DNA methylation, and down-regulate particular genes, according to many studies[48]. Regarding DNA damage induced by Na F, results of this current study showed significant increase in DNA tail length of Na F treated group. This was addressed by[49] who investigated the effects of Na F on the rat kidney and found that it caused DNA damage in the kidney by increasing comet length and tailing rate, and this damage was particularly visible after 4 to 6 weeks of exposure. Also,[50] described these findings in Red blood corpuscles of albino mice treated with sodium fluoride. Moreover, morphometric analysis in this study revealed some improvement in DNA damage when treating rats with Resveratrol along with Na F. This was described by[51] in a model of induced bronchial asthma through exposure to house dust mud (HDM) in C57BL/6J mice which showed improved DNA damage by comet assay on using Resveratrol.

On the other hand, in a study performed by[52]; Vitamin D3 was studied for its potential to protect against Na F-induced DNA damage in rat renal epithelial NRK-52E cells. It was stated that this harm was affected by both duration and level of exposure. After 3 hours, it was discovered that Na F caused DNA damage, and vitamin D3 was ineffective in mitigating the damage. At 12 and 24 hours, vitamin D3 decreased Na F-induced DNA damage.

Furthermore, when treating NRK-52E cells with Na F for different incubation periods,[53] discovered that all fluoride-treated groups had substantially more DNA deterioration and that when treating the cells with some antioxidants as vitamin E and Selenium in association with Na F, there was a substantial decline in DNA harm relative to those given only Na F.

CONCLUSION

According to the present evidence, fluoride has the ability to cause peroxidation in rats' kidneys, which can also result in degenerative changes and cell death. Resveratrol could protect rats' kidneys from fluoride-induced damage by increasing their antioxidant capacity. This investigation illustrated Resveratrol’s defensive impact over cellular peroxidation and DNA deterioration triggered by fluoride on rat renal tissue. In the future, resveratrol may be an important antioxidant to use in the treatment of fluorosis.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

اعتلال الحمض النووي والتغيرات الكيميائية الحيوية التي يسببها فلوريد الصوديوم على كلى الجرذان والتأثير المحتمل للريسفيراترول

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الخلفية: يعد الفلورايد عنصر نادر ومهم لجسم الإنسان وهو يستخدم على نطاق واسع في مجموعة متنوعة من الصناعات ، بما في ذلك المستحضرات الصيدلانية والزراعة والمبيدات الحشرية والمواد الخافضة للتوتر السطحي كما يعد الريسفيراترول الموجود في العنب والخوخ والتوت البري والفول السوداني من اهم مضادات الأكسدة القوية لأنه يقلل من الأضرار التأكسدية التي تسببها بعض المواد السامة في خلايا الحيوانات.

الهدف من البحث: يهدف هذا البحث الي معرفة مدى تأثير فلوريد الصوديوم على خلايا الكلى في الجرذان البيضاء والإنزيمات المؤكسدة والحمض النووي بها ودور عقار الريسفيراترول في الوقاية منه واصلاح التلف المصاحب له.

المواد والطرق المستخدمة: تم استخدام ٣٦ من ذكور الجرذان البيضاء في هذا البحث مقسمة إلى ٣ مجموعات رئيسية: المجموعة الضابطة ، المجوعة المعالجة التي تلقت فلوريد الصوديوم والمجموعة المحمية التي تلقت فلوريد الصوديوم مع عقار ريسفيراترول استمرت التجربة لمدة ٣٠ يومًا.تم صبغ عينات من الكلي بصبغة الايوسين والهيماتوكسيلين والماسون وقياس انزيمات الاكسدة بها مع عمل تحليل احصائي للنتائج.

النتائج: أظهر الفحص النسيجي للكلي في الجرذان المعالجة بفلوريد الصوديوم وجود فجوات بالخلايا مع نزيف فيما بينها واتساع انابيب الكلي واظهرت النتائج المورفومترية ازدياد سمك غشاء بومان المحيط بكبيبات الكلي كما كشفت البيانات البيوكيميائية عن ازدياد مستوي بعض الانزيمات وازدياد نسبة التلف في الحامض النووي وتم تحسن غالبية هذه المؤشرات في المجموعة التي تعالجت بالريسفيراترول وتحسنت الاضرار الناجمة عن فلوريد الصوديوم الي حد ما.

الاستنتاج: يمكن ان تستنتج من هذه الدراسة ان فلوريد الصوديوم بتركيزات عالية يؤثر سلبيا علي خلايا الكلي وتقل هذه التأثيرات باستخدام مضادات الاكسدة مثل الريسفيراترول.