

**Original article**



**Histopathological Alteration in the Spleen of Male Albino Rats Exposed to Sodium Fluoride with the Protective Role of Resveratrol**

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**ABSTRACT:**

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**Background:** Fluoride poisoning results from drinking fluoride rich ground water which is an important source of water supply throughout the world. Recently, resveratrol has been used as anti-oxidative, anti-inflammatory agent and it is a naturally occurring polyphenol present in grapes and red wine. **Aim of the work:** Evaluation of protective role of resveratrol against sodium fluoride (NaF) toxicity on the spleen. **Materials and methods:** 40 adult male albino rats were used in this work. The rats were divided into: Group I: This group consists of 10 rats

which did not receive any treatment. Group II: formed of 10 rats which were given 30 mg/kg B.W resveratrol once daily for 30 days orally by gastric tube. Group III: (NaF treated group): consists of 10 rats which were given 10mg/kg B.W of NaF dissolved in 2.5 ml distilled water once daily for 30 days orally by gastric tube. Group IV: (NaF and resveratrol treated group): formed of 10 rats which were given 10 mg/kg B.W of NaF and 30 mg/kg B.W of resveratrol once daily for 30 days by gastric tube. At the end of experiment the rats were sacrificed and peripheral blood samples were taken for neutrophils, monocytes and lymphocytes count and oxidative markers study then spleen tissues were taken for histo-pathological and immunohistochemical staining techniques. **Results:** NaF possessed immune toxic effect on the architecture of the spleen in the form of decreased the diameter and atrophy of lymphoid follicles of white bulb of the spleen and red bulb degeneration and these changes were improved after using the resveratrol. **Conclusion:** prolonged exposure to NaF can cause histopathological changes in the spleen structure which cause decreased in immune response and. resveratrol has the ability to ameliorate the changes which caused by NaF.

**Key words:** spleen; NaF; resveratrol; albino rat.

**1. INTRODUCTION:**

Fluoride found naturally in the water, soil, rocks, plants and air and it is also added in small amounts to public water supplies. This is called water fluoridation process and Fluoride is a mineral found in our bones and

teeth it is commonly used in dentistry to strengthen enamel. It is also used in medical imaging scans, such as Positron Emission Tomography (PET) Scan, as a cleaning agent, in pesticides and to make Teflon, steel, and aluminum products (Ozsvath., 2009).

Bone, teeth health and other essentials in medicinal chemistry need fluoride (Alcock., 1970, Hagmann., 2008, and Gillis., 2015). Drinking water naturally contaminated with fluoride induce fluorosis (Khan and Reddy ., 2014). And it also can transfer to the body through drugs, industrial emission, food and dental products (Ozsvath., 2009). Teeth, bones, and other organs will be in a deleterious condition if exposed to prolonged high concentration of fluoride (Olivier et al., 2010, Perumal., 2013 , Liu et al .,2014, Zhao et al., 2014 and Peng et al.,2016).

Previous researches have revealed that fluoride induces pathological injury and oxidative damage in the broiler chickens liver, kidney, and intestine (Gong et al.,2009, Bai et al 2010., Luo et al., 2013 a&b and Luo et al.,2016). Other studies indicated that cytotoxicity, apoptosis and DNA damage may be caused by the fluoride in human and animals (Song et al., 2002, Wang et al., 2004, Matsui., 2007, Ngoc., 2012 and Song et al.,2014).

It was reported that auto regulatory processes of inflammation and the immune system function may be disturbed by high fluorine intake (Fuente., 2016). Recent studies about mice cultured splenic lymphocytes showed reduction in cell proliferation and increase in cell apoptosis which caused by intake of excess sodium fluoride (Deng., 2016 and Kuang .,2016).

Wang et al., (2009) indicated that massive ingestion of fluoride seriously induced thymic apoptosis and damaged the immune function in female Wister rats. Exposure to high fluorine causes affection of immune organs like thymus gland (Chen., 2011), the

spleen (Chen et al., 2009), the bursa of Fabricius (Liu et al.,2012) and the cecal tonsil (Liu et al., 2012 and Liu et al., 2013) by decreasing the lymphocyte population, reduction of secretion of cytokines and increased apoptosis.

Drinking water contaminated with fluoride for long time may result in immune toxicity especially to spleen (Sosroseno., 2003and Giri et al., 2013).

Resveratrol is phytoalexin which is naturally present in red wine, grapes, grape juice, peanuts, cocoa and it has different biological activities as anti-oxidative, anti-inflammatory and anticarcinogenic (Sgambato et al., 2001). So in the present work we use resveratrol to prove its protective effect against the immune toxic effect of NaF on the spleen.

## **II. MATERIAL AND METHODS:**

### **II.1.Chemicals:**

- A. Sodium fluoride:** It was purchased from Al-Kahira Company for pharmaceutical industries as a powder and was dissolved in distilled water.
- B. Resveratrol:** It was purchased from United Arab Emirates manufactured by California Gold Nutrition as veggie capsules that were dissolved in distilled water.
- C. Distilled water:** It was brought from Kemecta Company.
- D. Enzyme-linked immuno-sorbent assay (ELISA) kits:** They were purchased from sigma, Aldrich, Germany and imported by Cairo

chemical company, Egypt for detection of the levels of catalase , glutathione peroxidase enzyme activity (GPx) and malondialdehyde (MDA) in the splenic tissue homogenate

- E. Dissection set, 10% formol saline, alcohol, xyelen and paraffin wax and microtome for light microscopic examination.

## II.2.Experimental animals:

40 adult male albino rats, aged 3-5 months, weighing 150-200 gm, were obtained and locally bred from the animal house, Faculty of Medicine, Zagazig University. They were housed in plastic cages at room temperature (20-26°C), and they had free access to commercial food pellets and water and kept under 12 Hr. light/dark cycles throughout the experiment. All experimental procedures were performed in accordance with the guidelines of Zagazig University Institutional Animal Care and Use Committee (ZU-IACUC).

### The rats were divided as follows:

- 1- **Group I: (negative control):** Consists of 10 rats which will receive only regular diet and tap water to measure the basic parameters.
- 2- **Group II: (positive control):** consists of 10 rats which will receive 30 mg/kg of resveratrol via gastric tube once daily for one month (**Sharma et al., 2018**).
- 3- **Group III: (NaF treated group):** consists of 10 rats which will receive 10mg/kg of NaF dissolved in 2.5 ml distilled water via gastric tube once

daily for one month (**Agustina et al., 2018**).

- 4- **Group IV: (NaF and resveratrol treated group):** consists of 10 rats which will receive 10 mg/kg of NaF and 30 mg/kg of resveratrol via gastric tube once daily for one month.

Day after administration of the last dose, a sample of each rat's blood about 1 ml was collected from the femoral vein of non-anesthetized rats (Horton et al 1986), Blood smears were prepared for differential counts. Then the rats were sacrificed by cervical dislocation and the spleen was dissected out carefully. Each spleen was dissected into two parts; one part was isolated in ice cold media for tissues homogenates preparation for determination of MDA, catalase and glutathione peroxidase. The other part was prepared for histo-pathological and immune-histochemical examination.

## II.3.Biochemical studies:

### Peripheral Blood Monocyte, Neutrophil, Lymphocyte Counts:

Leishman's stain was processed for each blood smear obtained from each animal. After drying, the average counts of peripheral blood monocytes, neutrophils and lymphocytes were determined by examination of the slides under the light microscope at 100X. The amount of monocytes, neutrophils, lymphocytes were counted per 100 leukocytes from each slide smear. Each type counted in the form of percentage for each type.

### Splenocytes Counts:

A forceps was used to take apart of isolated spleen sample in 50mg of tissue/5ml

of phosphate buffer solution (PBS) for splenocytes count by using a haemocytometer (Nandakar and Hegde., 1996). Then the results were assembled as number of splenocytes/ g splenic tissue.

#### **Oxidative and anti-oxidative Stress assessment**

Splenic tissues levels of Malondialdehyde (MDA) as a byproduct of lipid peroxidation, catalase and glutathione peroxidase (GPX) as antioxidants markers were measured according to instructions of commercial kits of Egypt Biodiagnostic.

Malondialdehyde (MDA) was assessed by spectrophotometer and the results were expressed as nmol/g tissue (Ohkawa et al., 1979).

Glutathione peroxidase (GPX) activity was assessed by spectrophotometer and the results were expressed as U/g protein (Sadasivam et al., 1996).

Catalase activity was assessed by spectrophotometer and the results were expressed as U/g protein (Beers and Sizer 1952).

#### **II.4.Histo-pathological studies:**

The splenic tissues were immersed in 10 % buffered formalin saline and processed for putting on in paraffin wax. Sections of 5- $\mu$ m thickness were obtained and prepared for the following stains:

Hematoxylin and Eosin (H&E) for indication of general histological structure of the spleen e.g: red pulp, white pulp and lymphoid follicles composition. (Drury and Wallington., 1980)

Masson trichrome stain for collagen fibers clarification (Chandler, 1933)

Immunohistochemical study for CD4 + immune cells: the sections were incubated with 1200/ CD4 for 1.5 hour at room temperature and then phosphate-buffered saline was used to rinse the slides then incubated with primary antibody peroxidase labeled dextran polymer conjugated with anti-mouse immune- globulins for one hour. Then the slides were incubated with secondary antibody 3,3 Diaminobenzidine for 15 mins, finally Myers hematoxylin was used as counter stain (Mohamed et al., 2017)

Stained sections were examined by using light microscope with an automatic photo micrographic camera system at histology department, faculty of medicine, Zagazig University.

#### **II.5.Morphometric study:**

Morphometric analysis was carried out in the Histology Department, Faculty of Medicine, Zagazig University using image analyzer Leica Q win V.3 program installed on a computer. Randomly five chosen fields in five chosen sections obtained from five different animals from the same group were used,

- White pulp lymphoid follicles diameter were measured by using (H&E) stained sections.
- Number CD4 positive immune cell in the red pulp were measured by CD4 immunohistochemically stained sections.
- Area % of collagen fibers were measured by using Masson's trichrome stained sections

## **II.6. Statistical Methods:**

All the data were analyzed using PSPP freeware with one-way ANOVA and Bonferroni Post Hoc T-test to indicate the level of significance between every two groups. When  $p$  is  $\leq 0.05$ , level of significance was obtained (Das and Das., 1998).

## **III. RESULTS:**

### **III.1. Biochemical results:**

The negative control and positive control groups showing nearly similar biochemical results. So, we compared the NaF treated group and NaF + resveratrol treated group with negative control group:

#### **Neutrophil, Lymphocyte, Monocyte and Splenocyte Count:**

By using T- test, there was significant decrease in lymphocytes, neutrophil and monocytes count in NaF treated group in relation to negative control group. On the other hand, there was non-significant difference in lymphocytes, neutrophil and monocytes count in NaF + resveratrol treated group as compared to control group as shown in (Table 1 and Figure 1).

As regarding the splenocyte count, there was significant decrease in Splenocyte count in NaF treated group in relation to the negative control group. On the other hand, there was non-significant difference in

splenocyte count in NaF + resveratrol treated as compared to control group as shown in (Table 1 and Figure 1).

#### **Oxidative Stress Analysis:**

By using T- test, there was significant decrease in catalase and peroxidase activity in NaF treated rats in comparison to negative control group. On the other side there was no significant difference in catalase and peroxidase activity in NaF + resveratrol treated group when compared to negative control group as shown in (Table 2 and Figure 2).

As regards the MDA activity, there was significant increase in MDA activity in NaF treated rats in comparison to negative control group. On the other side, there was no significant difference in MDA activity in NaF +resveratrol treated group when compared to negative control group (Table 2 and Figure 2).

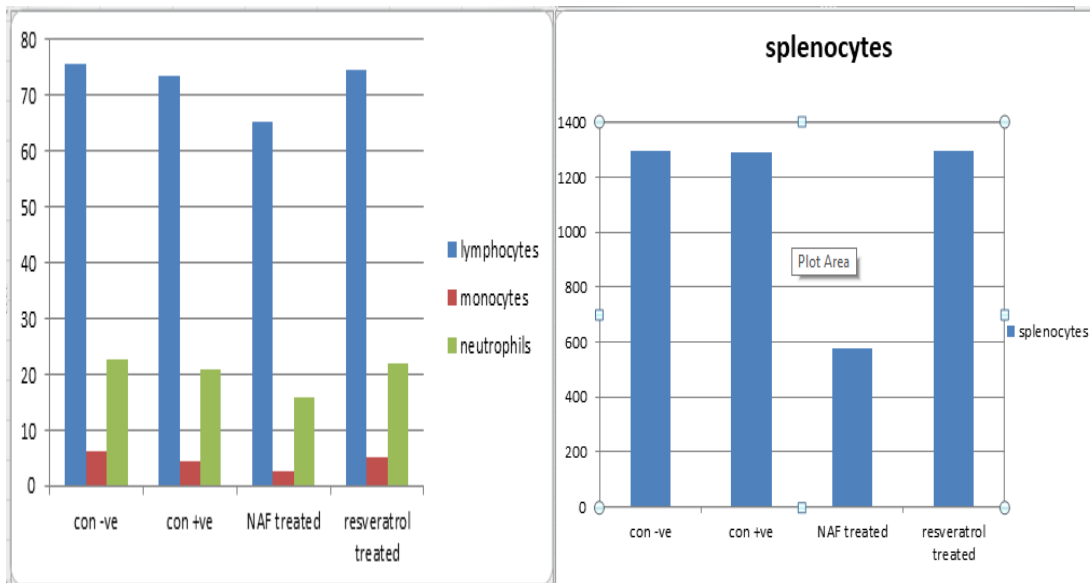
### **III.2. Histopathological results:**

The negative control and positive control groups showing nearly similar histological and immunohistochemical results. So, we compared the other groups with negative control group.

**Table 1:** Effect of NaF on lymphocytes, monocytes, neutrophils and splenocyte counts in different treated groups using Bonferroni Post Hoc T-test

| Groups<br>Parameters                                    | Negative control group<br>Mean ±SD | Positive control group<br>Mean ±SD | NaF treated group<br>Mean ±SD | NaF+resveratrol treated<br>Mean ±SD |
|---|------------------------------------|------------------------------------|-------------------------------|-------------------------------------|
| Lymphocytes (% of all leukocytes)                       | 75.50 ± 2.25                       | 73.55 ± 2                          | 68.19 ± 1.6**<br>P= 0.0008**  | 74.50 ± 2.23                        |
| Monocytes (% of all leukocytes)                         | 6.23 ± 0.64                        | 4.28 ± 0.61                        | 2.45 ± 0.84**<br>P= 0.0009**  | 5.24 ± 0.62                         |
| Neutrophils (% of all leukocytes)                       | 22.83 ± 0.76                       | 20.83 ± 0.74                       | 18.00 ± 0.42**<br>P= 0.0007** | 21.81 ± 0.75                        |
| Splenocyte counts (× 10 <sup>5</sup> /g splenic tissue) | 1296.43 ± 21.49                    | 1292.41 ± 21.47                    | 575.00 ± 20.1**<br>P= 0.001** | 1294.42 ± 20.48                     |

SD= standard deviation; NaF = sodium flouride; (\*\*): T-Test was significant when P value ≤ 0.05 as compared to negative control group.

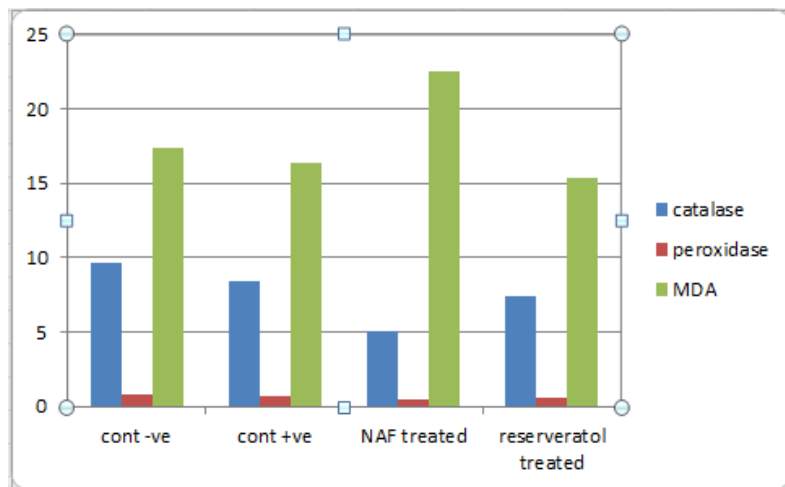


**Figure (1):** Bar chart for comparison between all treated groups all-over the period of the study as regards lymphocytes, monocytes, neutrophils and splenocyte counts.

**Table 2:** Effect of NaF on catalase and peroxidase activity and formation of malondialdehyde (MDA) in treated groups using Bonferroni Post Hoc T-test

| Groups<br>Parameters                      | Negative control<br>Mean ±SD | Positive control<br>Mean ±SD | NaF treated<br>Mean ±SD        | NaF+resveratrol treated<br>Mean ±SD |
|---|------------------------------|------------------------------|--------------------------------|-------------------------------------|
| Catalase (mM H2O2 consumed/mg tissue/min) | 9.47 ± 0.19                  | 8.47 ± 0.18                  | 5.10 ± 0.06**<br>P= 0.0009**   | 7.47 ± 0.16                         |
| Peroxidase Unit/mg tissue                 | 0.799 ± 0.008                | 0.699 ± 0.007                | 0.457 ± 0.013**<br>P= 0.0002** | 0.597 ± 0.007                       |
| MDA nM/mg tissue                          | 17.38 ± 0.32                 | 16.38 ± 0.31                 | 22.50 ± 0.28**<br>P= 0.0007**  | 15.38 ± 0.29                        |

SD= standard deviation; NaF = sodium flouride; MDA= malondialdehyde; (\*\*): T-Test was significant when P value ≤ 0.05 as compared to negative control group.



**Figure (2):** Bar chart for comparison between all treated groups all-over the period of the study as regards Catalase , peroxidase and MDA activity in splenic tissue.

**H&E stained sections:**

The H&E stained sections of positive and negative control groups showed the normal architecture of the spleen in the form of having distinct red pulp and white pulp (Figure 3A). The white bulb was formed from lymphoid follicles, the follicles were continuous with the per arterial lymphatic sheath (PALS) which surround eccentric arteriole, the lymphoid follicle consisted of lymphoid aggregation called mantle zone then germinal center and surrounded by marginal zone which is aggregation of lightly stained lymphocytes and separated white bulb from red bulb (Figure 3B). The red pulp showed many splenic cords with blood sinusoids in-between. Connective tissue capsule was covering the spleen (Figure 3c).

The H&E stained sections of NaF treated animals showed degeneration of lymphoid follicles forming white pulp which appeared atrophied and not surrounded by marginal zone moreover, the germinal center showed necrosis in some follicles and they were absent in other follicles (Figure 4 A&B&C). Relatively thick connective tissue capsule appeared in relation to control groups (Figure 4 D). Large congested sinusoids were also seen (Figure 4E). Lymphoid cells were degenerated with pyknotic nuclei and vacuolated cytoplasm. Megakaryocytes cells were frequently seen (Figure 4f).

The H&E stained sections of NaF+ resveratrol treated group showed great improvement in the architecture of the

spleen as it nearly restored the normal histological appearance in comparison to control groups in the form of intact white bulb and red bulb (figure 5 A) except some follicles still small in size and others large in size and some follicles also still losing the marginal zone (figure 5 B) with presence of some congestion in splenic sinusoids (Figure 5 c).

**Masson's trichome stained sections:**

In Masson's trichome stained sections of control group showed numerous thin collagenous fibers in the splenic capsule, trabaculea and around the wall of sinusoids (Figure 6 A&B).

In Masson's trichome stained sections of NaF treated group showed relatively thick collagenous fibers in the capsule, around sinusoids, within the splenic stroma (Figure 6 C).

In Masson's trichome stained sections of NaF + resveratrol treated group showed many collagen fibers appeared in the capsule, splenic stroma ,trabeculae and within the wall of sinusoids (Figure 6D).

**CD4 immunohistochemical stained sections:**

In CD4 immunohistochemical stained sections of control groups showed increased expression of CD4 positive Immune cell membrane mainly in the red pulp with some scattered cells in the germinal center of the white pulp (figure 7 A&B). In CD4 immunohistochemical stained sections of NaF treated groups showed decreased expression of CD4 positive immune cells in red pulp of the



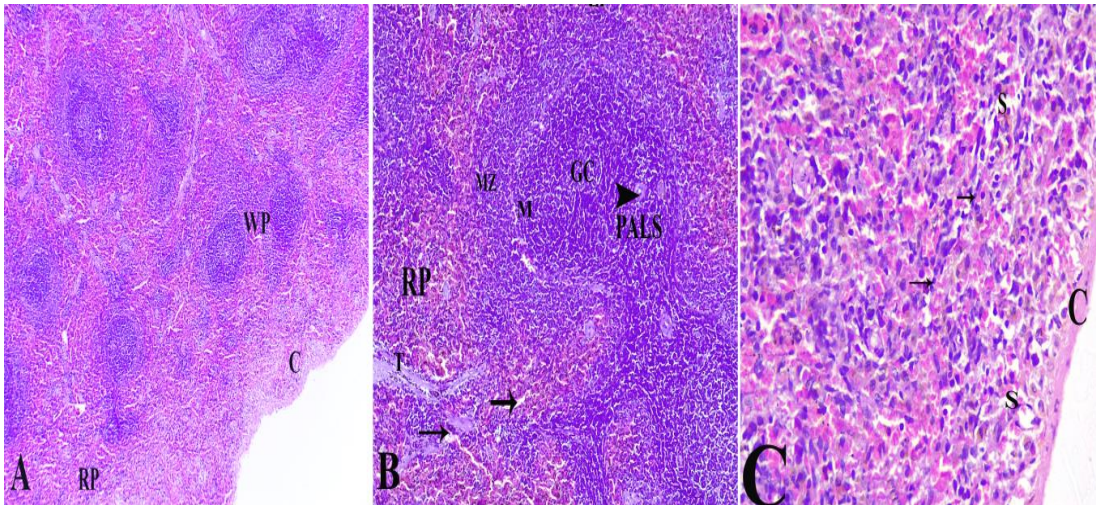
spleen (figure 7 C). In CD4 immunohistochemical stained sections of NaF+ resveratrol treated group moderate expression of CD4 positive immune cells in the red pulp of the spleen and in the germinal center of white pulp (figure 7D).

**III.3.Morphometric results**

The negative control and positive control groups showing nearly similar morphometric results. So, we compared the NaF treated group and NaF + resveratrol treated group with negative control group.

By using T test, there was significant decrease in lymphoid follicle diameter in NaF treated group as compared to control group. While, there was non-significant difference between NaF+ resveratrol treated group and control group as shown in (Table 3 and Figure 8). There was significant increase in area percent of collagen fibers in NaF treated group as compared to control group. While, there was non- significant difference between NaF +resveratrol treated group and control group as shown in (Table 3 and Figure 9).

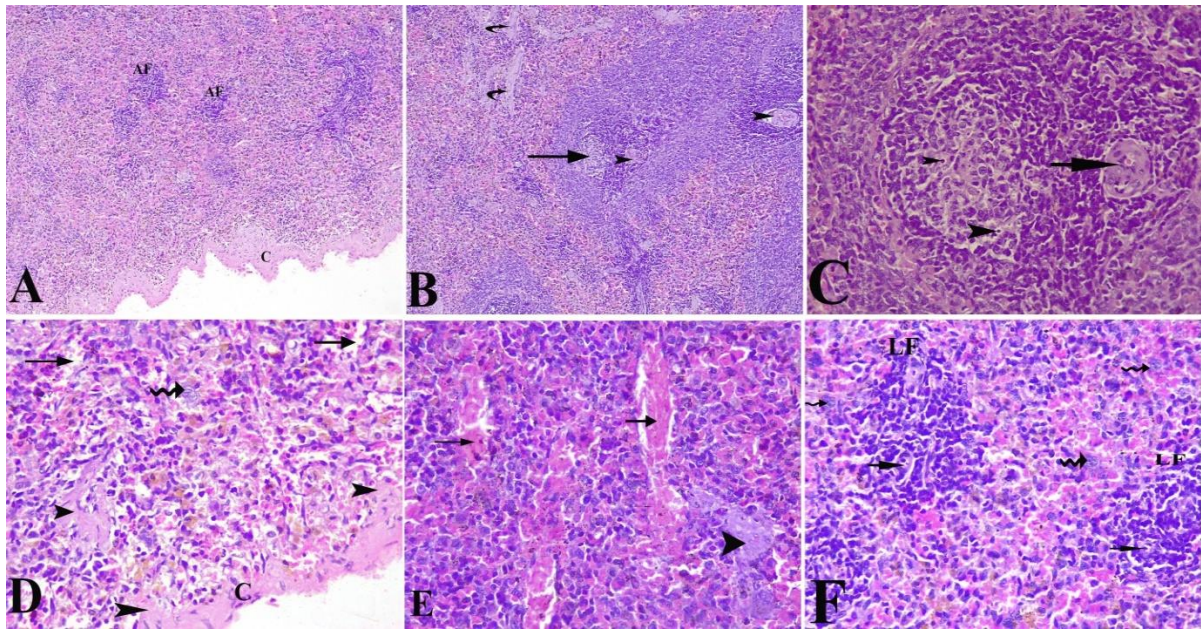
There was significant decrease in the number of CD4 positive immune cells in NaF treated group as compared to control group. While, there was non- significant difference between NaF + resveratrol treated group and control group as shown in (Table 3 and Figure 10).



**Figure (3):** (A): A photomicrograph of spleen of control groups showing that the spleen has intact white pulp (WP) and red pulp (RP) and surrounded by connective tissue capsule ( C ) (H&E x 40).

(B): A higher magnification of figure (A) showing the red pulp (RP) and the different layers of lymphoid follicles of the white pulp: marginal zone (MZ), mantle zone (M), germinal center (GC) and central arteriole (arrow heads) with peri- arterial lymphatic sheath (PALS) is also detected . Well organized trabacule (T) and intact thin walled sinusoids (arrows) were also detected (H&E x 100).

( C ): A photomicrograph of red pulp of spleen tissue of control group showing that the spleen is surrounded by connective tissue capsule ( C ) and splenic sinusoids (S) with splenic cords (arrows). No megakaryocytes were detected in red pulp of control group (H&E x 400).



**Figure (4): (A):** A photomicrograph of spleen tissues of NaF treated group showing loss of normal architecture of the spleen as there is loss of normal destination between white pulp and red pulp. The white pulp showing small sized, atrophied follicles (AF) and the spleen is surrounded by relatively thick capsule ( C ) (H&E x40).

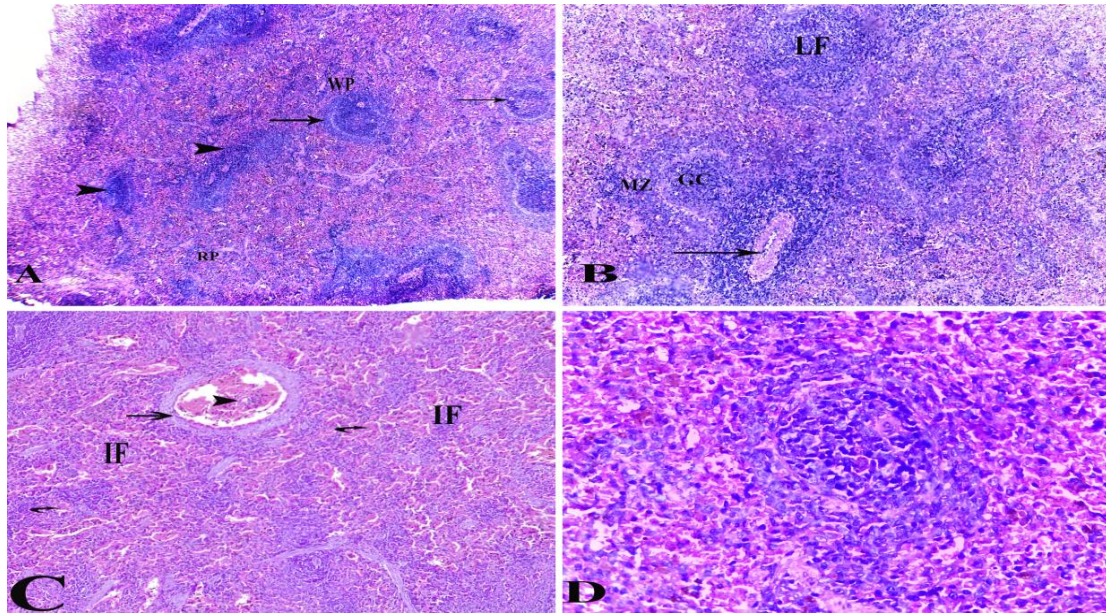
**(B):** A photomicrograph of spleen tissues of NaF treated group showing that the atrophied follicles shows necrosis in its center (arrow) , congested thickened wall central arteriole (arrow head) and disturbed trabeculae (curved arrow) (H&E x 100 ).

**( C ):** A higher magnification of figure B showing necrosis in the center of lymphoid follicles with the lymphocytes cells appear with vacuolated cytoplasm and pyknotic nuclei (arrow head) and congested central arteriole (arrow) (H&E x 400 ).

**(D):** A photomicrograph of spleen tissues of NaF treated group showing thickened dispersed connective tissue capsule (C) with thickened disturbed trabaculae (arrow head) and dilated sinusoids (arrows). Megakaryocyte cell is also noticed (wavy arrow) (H&E x 400).

**(E):** A photomicrograph of spleen of NaF treated group showing sever congestion, dilatation and hemorrhage of sinusoids (arrow) with dilated disturbed trabecular vein ( arrow head) (H&E x 400 ).

**(F):** A photomicrograph of spleen tissues of NaF treated group showing lymphoid follicle (LF) with disturbed structure. Lymphoid cells were degenerated with pyknotic nuclei and vacuolated cytoplasm (arrows). Megakaryocytes cells (wavy arrow) were frequently seen (H&E x 400).

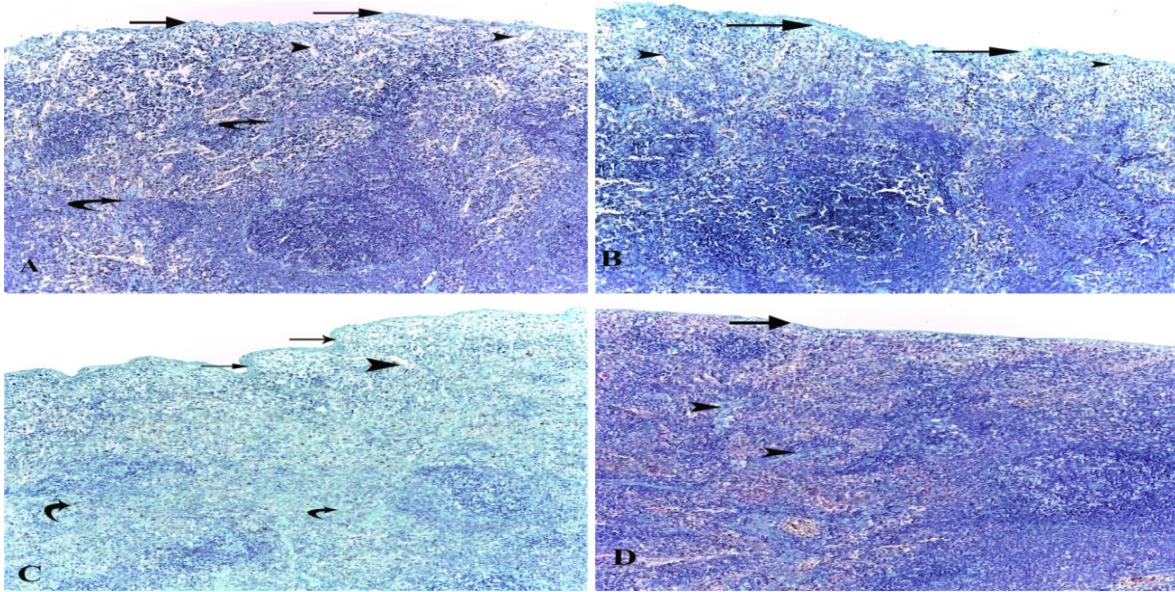


**Figure (5): (A):** A photomicrograph of NaF + resveratrol treated group showing that the spleen nearly retained its normal architecture in the form of red pulp (RP) and white pulp (WP). The lymphoid follicles may still with small sizes and atrophied (arrow heads) or retained its normal structure (arrows) (H&E x 40).

**(B):** A photomicrograph of NaF + resveratrol treated group showing lymphoid follicles of white pulp (LF) which lose its marginal zone and the other one has marginal zone (MZ) , germinal center (GC) and dilated central arteriole (arrow) (H&E x 100 ).

**( C ) :** A photomicrograph of NaF + resveratrol treated group showing thick walled dilated blood vessel (arrow) with hemorrhage at its center (arrow head). Dilated congested sinusoids (curved arrows) and cellular infiltrates (IF) also detected. (H&E x 400).

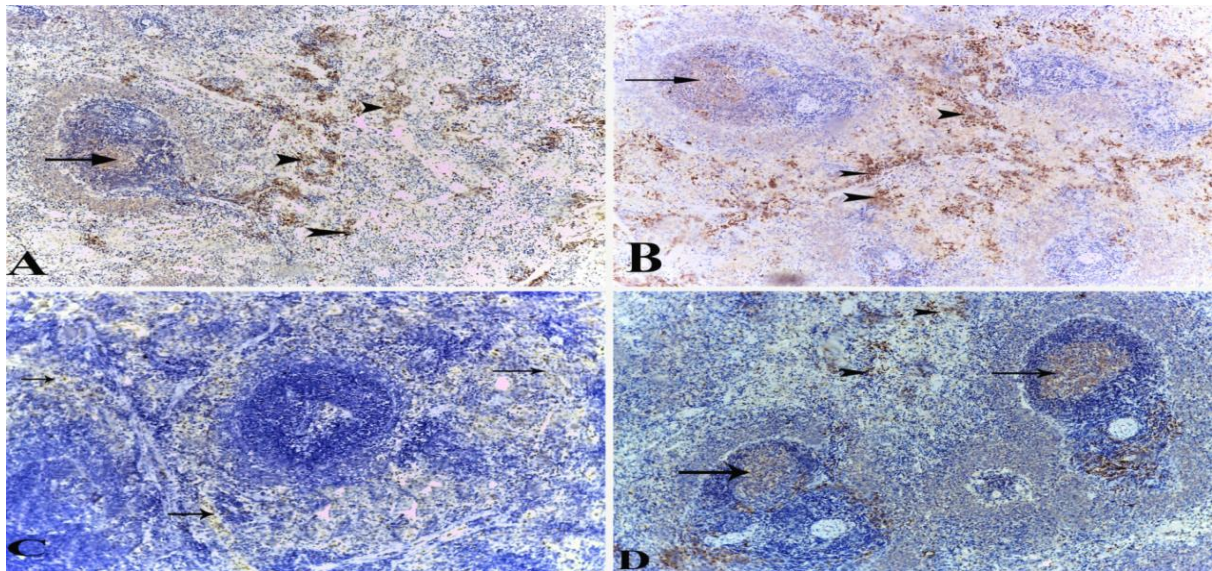
**(D) :** A photomicrograph of NaF + resveratrol treated group showing no megakaryocytes cells in the red pulp (H&E x 400 ).



**Figure (6): (A&B)** photomicrographs of Masson Trichrome stained sections of control group showing thin blue color in connective tissue capsule (arrows) surrounding the spleen with few blue collagen fibers through the sinusoids (arrow heads) and trabeculae (curved arrow) (Masson Trichrome X 100).

**(C):** A photomicrograph of Masson Trichrome stained sections of NaF treated group showing thick connective tissue capsule (arrows) with abundant blue collagen fibers through the sinusoids (arrow heads) and stroma (curved arrow) (Masson Trichrome X 100).

**(D):** A photomicrograph of Masson Trichrome stained sections of NaF + resveratrol treated group showing thin connective tissue capsule (arrows) surrounding the spleen with few blue collagen fibers through the trabeculae (arrow heads) (Masson Trichrome X 100).



**Figure (7): (A&B):** photomicrographs of CD4 immunohistochemical stained sections of control groups showed increased expression of CD4 positive Immune cell membrane mainly in the red pulp (arrow head) with some scattered cells in the germinal center of the white pulp (arrow) (CD4 immunohistochemical X100).

(C): A photomicrograph of CD4 immunohistochemical stained sections of NaF treated groups showed decreased expression of CD4 positive immune cells in red pulp of the spleen (arrows). (CD4 immunohistochemical X 100).

(D): A photomicrograph of CD4 immunohistochemical stained sections of NaF+ resveratrol treated group showed moderate expression of CD4 positive immune cells in the red pulp of the spleen (arrow head) and in the germinal center of the white pulp (arrow) (CD4 immunohistochemical X100).

**Table 3: Comparing the mean white pulp lymphoid follicles diameter, the number CD4 positive immune cell in the red pulp and the Area % of collagen fibers in different studied groups using Bonferroni Post Hoc T-test:**

| Groups / Parameters                             | Negative control Mean ±SD | Positive control group Mean ±SD | NaF group Mean ±SD          | NaF+resveratrol Mean ±SD |
|---|---------------------------|---------------------------------|-----------------------------|--------------------------|
| white pulp lymphoid follicles diameter          | 43.5 ± 4.0                | 41.4 ± 4.0                      | 18.9 ± 2.3**<br>P= 0.0002** | 40.4 ± 3.0               |
| Area % of collagen fibers                       | 55.3± 2.1                 | 53.4 ±2.0                       | 75.2 ± 2.4**<br>P= 0.0004** | 52.4 ±2.1                |
| number CD4 positive immune cell in the red pulp | 67.5 ± 8.1                | 66.4 ± 7.9                      | 10.8 ± 2.8**<br>P= 0.001**  | 64.1 ±7.6                |

SD= standard deviation.

NaF = sodium flouride.

(\*\*): T-Test was significant when P value ≤ 0.05 as compared to negative control group

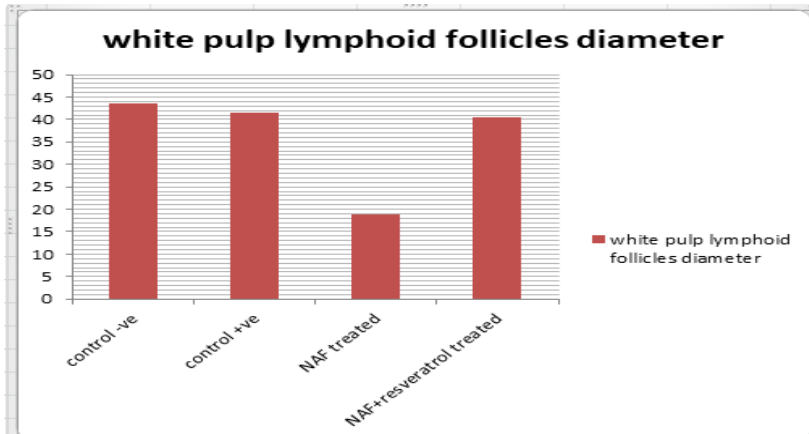


Figure (8): Bar chart for comparison between all treated groups all-over the period of the study as regard the morphometric results of the mean diameter of white pulp lymphoid follicles in µm.

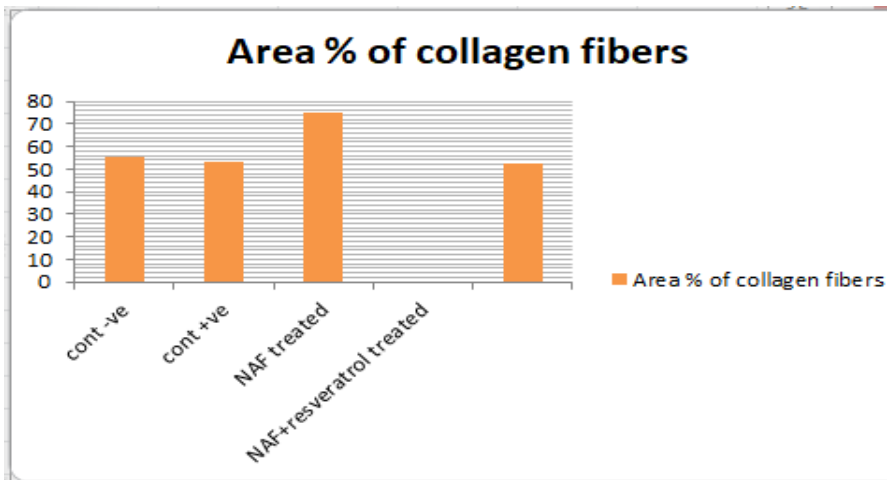


Figure (9): Bar Chart for comparison between all treated groups all-over the period of the study as regards the morphometric results of the mean percent area of collagen fibers.

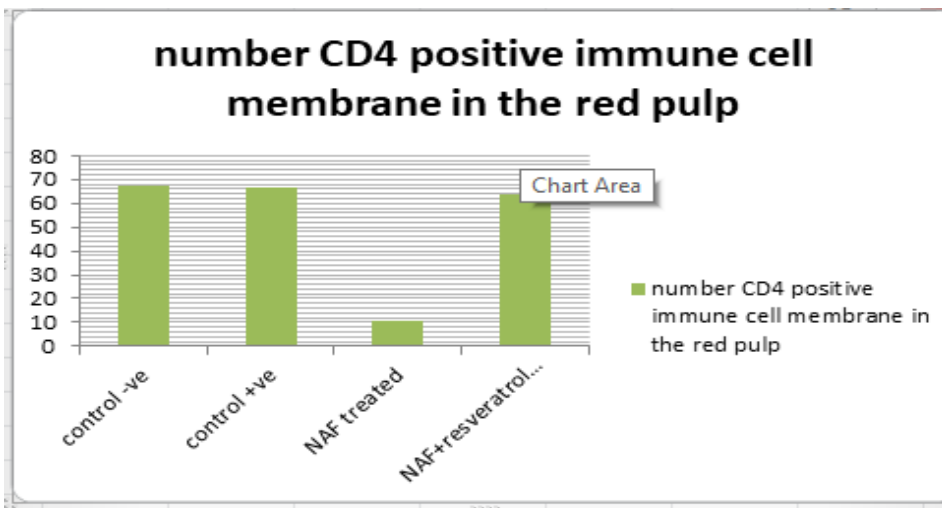


Figure (10): Bar Chart for comparison between all treated groups all over the period of the study as regards the morphometric results of the mean number of CD4+ immune cells in the red bulb in treated groups.

#### **IV. DISCUSSION:**

Fluorides are well known disruptive toxins, Accumulation in the body causes apoptosis in spleen as reported by Vilber et al. (1990) and Chen et al. (2009).

As regarding the control groups in current work, the sections stained with Hematoxyline & Eosin showed the normal architecture of the spleen in the form of having distinct red pulp and white pulp. Splenic cords appeared in red pulp. It showed well developed lymphoid follicles with eccentric arteriole, marginal zones circumscribed the lymphoid follicles and separated them from the red pulp, circular band of marginal sinuses separated the follicles from marginal zones and these results were in agreement with Arbind and Sapna .,(2013).

As regarding NaF treated group their stained sections showed loss of normal architecture of spleen as it showed small sized atrophied lymphoid follicles which

antioxidants and these results were in agreement with Das et al.,(2005) who attributed these changes to oxidative stress damage.

One of the observed results in the present study in NaF treated group, there was increased lymphocytes infiltrates from white pulp to red pulp and these results were in agreement with Hui et al., (2007) who explained that NaF increased intracellular free calcium amount that plays a principle role in fluorosis renal injury and increased the activity of Ca ATPase which cause disruption in the cell-cell and cell matrix

form the white bulb of the spleen with absence of line of demarcation between white bulb and red bulb. The lymphocytes of the lymphoid follicles appeared with vacuolated cytoplasm and pyknotic nuclei. White pulp atrophy was implemented with red pulp compensatory mechanism which is megakaryocytes proliferation as a result of apoptotic degenerative changes and these results were in agreement with Ajibade et al. (2015).

Large congested venous sinusoid was seen with thick irregular trabeculae and these results were in line with Machalinska et al., (2002) and Podder et al., (2010). These results were concomitant with decreased the monocytes, neutrophils, lymphocytes and splenocytes count, decreased the splenocytes count reflected the organ cell damage due to increased oxidative stress and decreased level of

adhesion, leading to red bulb infiltrates with lymphocytes.

Wójciak-Stothard et al., (2001) said that drinking water contaminated with fluoride for long time caused alteration in human red blood cells due to increase the level of phospholipids, cholesterol and lipid peroxidation and may induce alteration in red cell membrane biochemical compositions by producing free radicals and there by formation of abnormal erythrocytes.

As regarding the sections of control group stained with Masson Trichrome



indicated that collagen fibers showed normal arrangement in the capsule and in the extending trabaculae. On the other side the mean percent area of collagen fibers in the NaF treated group showed significant increase in comparison to control group and these finding proved by morphometric analysis and these results were in line with Fayza et al.,(2014) who said that there was significant increase in collagen fibers percent area in rat lungs treated with NaF and also these results were in agreement with Shashi1 and Manisha., (2021) who showed that adrenal gland of NaF treated rat showed that adrenal tissue was decellularized with increased collagen deposition. The induction of collagen synthesis was due to increased secretion of growth factors and liberation of cytokines by leukocytes (Castejón and Arismendi., 2006).

As regarding immune-histochemical stained sections for CD4+immune cells, the NaF treated rats showed significant reduction of CD4+ immune cells in comparison to control group which was proved by morphometric analysis results and these results were in agreement with Fuente et al., (2016) who proved that excess exposure to NaF cause significant reduction in the mean percentages of CD4+ and CD3+ T lymphocytes of the spleen.

CD4 present on some immune cell membranes like macrophages and T-helper cells as a glycoprotein and named the cluster of differentiation 4. Macrophages are giant immune cells which have both phagocytic and antigen

presenting functions that are essential for both adaptive and innate immune responses. T-helper lymphocytes have a principle role in adaptive immune response. After antigen recognition and TH cell recruitment, naive TH cells are activated and differentiated into cells capable of cytokine secretion to promote and steer the immune response (Hesketh et al., 2017 and Van den Broek et al., 2018).

As regarding NaF+ resveratrol treated group, the H&E stained sections showed great improvement in the architecture of the spleen as it nearly restored the normal histological appearance in comparison to control groups and these results were proved morphometrically as there was non-significant difference in the size of white bulb lymphoid follicle , collagen fibers mean percentage area and mean number of CD4+ immune cells between resveratrol treated group and control group and also improvement of the numbers of peripheral blood cells and numbers of splenocytes. Decreased MDA levels in NaF+ resveratrol treated group was due to its antioxidant activity and these results were said by Karabulut et al., (2006) who reported that resveratrol caused decrease in MDA levels in splenic tissues of rats exposed to hepatic ischemia-reperfusion (I/R) and it was due to its antioxidant effects through decreasing the liberation of free radicles.

As many lymphocytes pass through the spleen every day more than other lymphoid tissue, it makes the spleen the

most essential secondary lymphoid organ in lymphoid system (Bajénoff et al., 2008). Normal histological structure of spleen as secondary lymphoid organ is important for intact infection control by immune system that allow proper scavenging of pathogens and activating adaptive immunity (Borges da Silva et al., 2015). Any structural or functional affection of spleen that lead to dysfunction of immune system is called immunotoxicity which occurred if there is impairment in T-lymphocytes (cellular immunity) or B-lymphocytes (humoral immunity) (Shieh et al., 2014).

Resveratrol is well known to have antioxidant potency depending on antioxidant enzymes activation (De la Lastra and Villegas 2007). In our results, resveratrol markedly reduced oxidative stress through reducing catalase and peroxidase content in splenic tissue treated with it and these results were in agreement with Chih-Chun et al., (2012) who reported that resveratrol had the ability to prevent protein oxidation and decrease superoxide anion expression in the splenic tissues of STZ-induced diabetic rat and act as scavenger for free radicals.

**V. CONCLUSIONS:**

Administration of NaF caused immune toxic effect on the spleen as it causes loss of normal architecture of the spleen in the form of loss of normal appearance of white pulp and red pulp with disturbed capsule ,trabaculae and sinusoids and also it caused decrease in lymphocytes ,monocytes, neutrophils

and splenocytes counts with increased the level of MDA and decreased the level of catalase and peroxidase activities and resveratrol can ameliorate its toxicity on the spleen of male albino rats by reversing all the previous changes as it possess potent antioxidant action

**.VI. RECOMMENDATIONS:**

Further studies on adult male spleen with high doses of NaF exposure with more investigations for immune system are recommended. Further studies must be done about fluoride usage, further molecular studies to know more about mechanisms of toxicity and mechanisms of resveratrol protection

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**VIII. CONFLICT OF INTEREST:**

There are no conflicts of interest.

**VIII. REFERENCES:**

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## الملخص العربي

التغيرات النسيجية المرضية في طحال ذكور الجرذان البيضاء الذين تعرضوا لمادة فلوريد الصوديوم مع الدور الوقائي لمادة الريزفيراترول

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**المقدمة:** ينشأ تسمم الفلورايد من شرب المياه الجوفية الغنية بالفلورايد والتي تعتبر مصدر مهم لإمداد الماء في كل العالم. حديثاً، تستخدم مادة الريزفيراترول كمادة مضادة للأكسدة ومضادة للالتهابات وكذلك مضادة لمرض السكر ومادة الريزفيراترول مادة طبيعية موجودة في العنب و النبيذ الأحمر. **الهدف:** هدف هذا العمل إلى دراسة الآثار المترتبة على إستخدام مادة فلوريد الصوديوم على طحال ذكر الجرذ الأبيض البالغ والدور الوقائي لمادة الريزفيراترول. **المواد والطرق:** تم استخدام أربعين ذكراً من الجرذان البيضاء البالغة في هذه الدراسة، الذين تتراوح أعمارهم بين 4-6 أشهر، وتتراوح اوزانهم من 150-180 جم. تم تقسيم الجرذان بالتساوي إلى أربعة مجموعات: المجموعة الأولى: الضابطة السلبية وتحتوى على 10 فئران والتي لم تتناول شيئاً. المجموعة الثانية: الضابطة الايجابية والتي تلقت 30 مل من مادة الريزفيراترول بالفم يومياً لمدة ثلاثون يوماً. المجموعة الثالثة: المجموعة المعالجة بمادة فلوريد الصوديوم والتي تلقت 10 مجم لكل كجم مذابة في 2.5 مل من الماء يومياً لمدة ثلاثون يوماً المجموعة الرابعة: والتي تلقت مادة فلوريد الصوديوم كالمجموعة السابقة مصحوبة ب 30 ملجم من مادة الريزفيراترول. **النتائج:** كشف العمل الحالي أن إعطاء مادة فلوريد الصوديوم قد تسبب في حدوث تغييرات هيكلية كبيرة في طحال الجرذان في المجموعة الثالثة والتي تسببت في فقدان التمييز بين اللب الأبيض واللح الأحمر مع بصيالات لمفاوية غير محددة و إحتقان في الأوعية الدموية و إرتفاع نسبة المواد المضادة للأكسدة وإنخفاض عدد خلايا الطحال وإنخفاض عدد الخلايا المكونة للدم وكذلك بينما ظهر تحسن ملحوظ في التركيب النسيجي للطحال في المجموعة التي تلقت مادة الريزفيراترول حيث ظهر اللب الأبيض و اللب الأحمر بشكل طبيعى مع إنخفاض نسبة المواد المضادة للأكسدة وإرتفاع عدد خلايا الطحال وكذلك إرتفاع عدد الخلايا المكونة للدم. **الخلاصة:** تسبب مادة فلوريد الصوديوم في تغيير هيكلية في تركيب الطحال للجرذان وكان التغيير كبيراً ولكنه تم التحسن مع إستخدام مادة الريزفيراترول.