# The Cytotoxic and Ultrastrctural Perturbations of Aluminum Exposed Nile Catfish with Special Reference to the Mitigating Effect of Vitamin C

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**Abstract:** The genotoxic parameters are currently among the most valuable fish biomarkers for environmental risk assessment. So, the present study was directed to explore the toxic impacts of Aluminum chloride ($AlCl_3$) on DNA damage, apoptosis, antioxidant status and ultrastructural investigations of Nile catfish. The experiment was carried out on 48 fish that randomly divided into four equal groups with two replicates; the first group exposed to 1/20 $LC_{50}$ of $AlCl_3$, the second group exposed to the combined doses of $AlCl_3$ (1/20 $LC_{50}$) and vitamin C (Vit. C) at dose of 5 ppm, the third group exposed to Vit C at the dose previously mentioned and the fourth group was kept as negative control. The experiment was terminated after six months where the fish were sacrified and specimens from liver and gills of all groups were obtained and kept at -20°C till applying the required measurements and another specimen from the same organs were fixed in 10% neutral –buffered formalin and 3% glutaraldehyde solution for histopathological examination. The results indicated pronounced significant increase in malondialdehyde (MDA) concentration and significant decrease in both reduced glutathione (GSH) concentration and superoxide dismutase (SOD) and catalase (CAT) enzyme activity. $AlCl_3$ elicited an obvious increase in oxidative DNA damage and frequency of apoptotic cells, these manifestation were markedly ameliorated in the group exposed to the combined doses of $AlCl_3$ and Vit C. The ultrastructural histopathological findings proved the aforementioned results. It could be concluded that $AlCl_3$ elucidated a marked ruinous effects on the oxidative and genotoxic impacts as well as the histopathological alterations which were alleviated by Vitamin C.

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**Key words:** Aluminum chloride, Apoptosis, DNA damage, Oxidative stress, Ultrastructural investigations**,** Vitamin C.

## 1. Introduction

In aquatic ecosystems, the levels of toxic metals have increased either directly, as a result of atmospheric deposition, waste –water discharge and run off (eg., Pb, Hg, Cu and Zn) or indirectly, through increased solubilization and mobilization from sediments (eg., Fe and Al). Diverse studies had demonstrated that Aluminum (Al) is toxic to aquatic fauna, especially in fishes on which may produce gill damage due to metal deposition and changes in osmoregulation as well as oxidative stress in lymphocytes **(Garcia-Medina *et al.*, 2010).**

In last years the problems of the drainage canals in Egypt have extremely increased. These problems include the presence of high concentrations of different metals and pesticides in both water and various fish organs **(Khallaf *et al.*, 2003 and Authman *et al.*, 2008)**. As a result, fish are exposed to water that contains high concentrations of metals including aluminum **(Authman, 2011).**

Aluminum (Al) is the third most common and ubiquitous element on earth□s lithosphere after oxygen and silicon **(Camargo *et al.*, 2009)**. This metal has numerous applications including most notably the manufacture of kitchen utensils and food and drink packaging, the production of dyes, baking powder and antiacids and as an anticoagulant of organic matter in water purification **(Atli *et al.*, 2006).** Al toxicity are restricted to fish species from the Northern hemisphere **(Monette and McCormick, 2008)**. In the tropical and neotropical areas such studies are still rare **(Barcarolli and Martinez, 2004)**. Physiological alterations frequently observed in different fish species exposed to Al are mainly related to disturbances, beyond structural gill damage **(Peuranen *et al.*, 1993)**, cardiovascular (**Laitinen and Valtonen, 1995)**, hematologic **(Barcarolli and Martinez, 2004)**, respiratory, ionoregulatory **(Poléo, 1995)**, reproductive **(Vuorinen *et al.*, 2003)**, metabolic **(Brodeur *et al.*, 2001)**, and endocrine **(Waring *et al.*, 1996)**.

The liver is the main and important detoxifying organ in fish and is essential for both the metabolism and the excretion of toxic substances in the body **(van Dyk *et al.*, 2007)**, and the gills are a multi-functional organ playing an important role in osmoregulation of fish **(Hwang and Lee, 2008)**. Al binds to the gills of many species such as Atlantic Salmon *(Salmo salar)* and brook Trout *(Salvelinus*

*fontinalis)* **(Smith and Haines, 1995)**; which leading to ionoregulatory and respiratory stress **(Bonga, 1997)**. In addition it induces damage at gill level due to increased mucus production which alters osmoregulation and respiratory processes **(Ward *et al.*, 2006)**.

In order to assess effects of environmental pollutants on aquatic ecosystems, there is a suite of fish biomarkers which may be examined. The analysis of DNA alterations in aquatic organisms has been shown to be a highly suitable method for evaluating the genotoxic impacts of environments. The comet assay has wide application as a simple and sensitive method for evaluating DNA damage in fish exposed to various xenobiotics in the aquatic environment **(Frenzilli *et al.*, 2009).** In regard to aquatic organisms, studies on the geno/cytotoxic effects of Al are scarce. However, in the larvae of the *dipteran Chironomus riparius*; diverse chromosomal aberrations were demonstrated **(Michailova *et al.*, 2003)**; in the hemocytes of the *leech Hirudo verbena* and in erythrocytes of the mosquitofish *Gambusia holbrooki*, authors have found an increase in DNA damage measured with the comet assay **(Kovačević *et al.*, 2009 and Ternjej *et al.*, 2010).** Morever, most studies on the genotoxic and cytotoxic potentials of Al have been made *in vitro*. In cultured human lymphocytes, **Banasik *et al.* (2005)** demonstrated an increase in the number of apoptotic cells. Also, Al is considered to be a non-redox active metal, it promotes biological oxidation both *in vitro* and *in vivo* **(Turner and Lysiak, 2008).**

Some vitamins contribute to the detoxification process from reactive oxygen species (ROS) such as ascorbic acid, which is considered as the most water soluble antioxidant in extracellular fluids **(Blokhina *et al.*, 2003)**. The molecular mechanisms of the antiscorbutic effect of Vitamin C are largely, although not completely understood **(Carr and Frei, 1999 and Yousef, 2004).**

In the light of the above and because there is paucity of reports concerning Al impacts in catfish, this study aimed to clarify DNA damage and apoptotic effect by using comet assay and flow cytometry, also to elucidate the level of antioxidant enzymes activity and ultrastructural perturbations using electron microscopy technique in liver and gills with special reference to vitamin C in mitigating these effects.

## 2. Material and methods

### Fish and experimental protocol

A total number of forty eight Nile catfish with an average body weight ranged from 140-160 gm were used in the present study. Fish were obtained from Abbassa fish Hatchery at Sharkia Province, Egypt. Fish were apparently healthy and free from skin lesions or external parasites, they were maintained in glass aquaria (80x 40x 30 $cm^3$ capacity for each) having 96 liters of dechlorinated tape water. Each aquarium provided with aerator, thermostatically controlled with heater and thermometer. Fish were acclimatized for two weeks to laboratory environment. They were fed with basal diet 35.4 % crude protein, the amount of food per day (on dry matter basis) was 3% of fish body weight and fed three times daily. Fish were randomly divided into four equal groups each of two replicate (six fish each).

The fish of first group exposed to Aluminum chloride ($AlCl_3$) at a dose of 1/20 $LC_{50}$ according to **Hamed (2012)**; the second group exposed to both combination of $AlCl_3$ at the same dose previously mentioned and vitamin C (Vit C) at dose of 5 ppm in the water **(Kumar *et al.*, 2009),** the third group exposed to the dose of Vit C lonely and the fourth group was kept as negative control. The experiment was terminated after six months.

### Chemicals

Aluminium chloride ($AlCl_3$) and vitamin C (Vit C) used in the present experiment were purchased from El- Gomhoria Chemical Company. Egypt.

### Sampling and measurements

At the end of experiment, fish were sacrificed by decapitation and dissected. Specimens from liver and gills of both treated and control groups were obtained and kept at -20°C till applying the required antioxidant measurements and both comet and flow cytometry assays. Another specimen from the same organs of all groups preserved in 10% neutral –buffered formalin for histopathological examination, also very small specimens were fixed by immersion in 3% glutaraldehyde solution for ultrastructural investigation.

### Evaluation of oxidative stress

### Preparation of tissue Homogenate

About one gram of the liver and gills were homogenated with 9.00 ml potassium phosphate buffer solution pH 7.40, then briefly solicited and centrifuged at 3000 rpm for 15 min. the supernatant was separated and used freshly for estimating the biochemical assays of antioxidant activity.

### Determination of lipid peroxidation (Measurement of malondialdehyde; MDA level)

Lipid peroxides in both liver and gills homogenates were ascertained by measuring malondialdehyde (MDA) through thiobarbituric acid method **(Ohkawa *et al.*, 1979).**

### Determination of reduced glutathione (GSH) concentration

GSH was estimated in liver and gills homogenates according to the method described by **Beutler *et al.* (1963)**

### Determination of Superoxide dismutase (SOD) activity

SOD activity was estimated in liver and gills homogenates according to the method of **Misra and Fridovich, (1972).**

**Determination of Catalase (CAT) activity**

CAT activity was determined according to the method of **Sinha (1972)**.

**Single cell gel electrophoresis (SCGE); Comet assay**

Liver and gills pieces of the treated and control groups were placed into a small Petri dish with ice-cold mincing solution ($Ca^{2+}$- and $Mg^{2+}$-free HBSS containing 20 mM EDTA and 10% DMSO). The viability of the cells of both examined organs was indirectly determined by analyzing the comet images after electrophoresis **(Endoh *et al.*, 2002)**. The samples were cut into smaller pieces, using a disposable microtome razor blade, and the solution was aspirated. Then, a fresh mincing solution was added and the samples were minced again to finer pieces. Resulting cell suspensions were collected and filtered (100 µm nylon meshes). All samples were stocked on ice in appropriate conditions to avoid light until the comet assay procedures.

The Comet assay was performed under alkaline conditions according to a previously described standard protocol **Collins and Dunsinka (2002)**. Briefly, an aliquot of 5 µl of each prepared cell suspension was mixed with 120 µl of 0.5% low melting point agarose at 37°C and layered onto conventional microscope slides, precoated with 1.5% normal melting point agarose. The slides were placed overnight in freshly prepared cold lysing solution (1% Triton X-100, 2.5 mM NaCl, 0.1 mM $Na_2EDTA$, 10 mM Tris with 10% DMSO, pH 10.0) and then in a horizontal electrophoresis cube with alkaline electrophoresis solution (0.3 M NaOH, 1 mM $Na_2EDTA$, pH >13) at $4^oC$ for 20 min. The electrophoresis was performed at 25 V and 300 mA for 20 min. After electrophoresis, the slides were washed twice for 5 min in neutralizing buffer (0.4 M Tris-HCl, pH 7.5), fixed for 5 min in absolute alcohol, air-dried, and stored at room temperature. In order to evaluate extremely low molecular weight DNA diffusion, two slides from each animal were removed after lysis procedure, rinsed with neutralizing solution, fixed and air-dried, and stored until analysis.

Immediately before analysis, the DNA was stained with 50 µl of 20 µg/ml ethidium bromide. The slides were examined with a 40X objective lens with epi-illuminated fluorescence microscopy (Olympus-Bx60, excitation filter: 515-560 nm; barrier filter: 590 nm) attached to a color CCD video camera and connected to an image analysis system (Comet II, Perspective Instruments, UK). The Comets were analyzed by a visual scoring method and computerized image analysis (**Liu *et al.*, 2002)**. To quantify DNA damage, tail length (TL), tail DNA (%) (TDNA) and tail moment (TM) were analyzed using Comet Assay Project Software (CAPS), generally 50-100 randomly selected cells are analyzed per sample.

**Flow cytometry assay of apoptosis**

Tissue specimens were prepared as follow, the specimens were washed with isotonic tris EDTA buffer, 3.029 gm of 0.1 M tris (hydroxymethyl amino methane, 1.022 gm of 0.07 M sodium chloride and 0.47 gm of 0.005 M EDTA), then dissolved in 250 ml of distilled water and then adjust the pH at 7.5 by using 1 N HCl. The cell suspension was centrifuged at 1800 rpm for 10 min., where upon the supernatant was aspirated. If they were macroscopically contaminated with blood, it was then subjected to hemolysis with filtered tape water for 10 min., after centrifugation and aspiration of the supernatant the cell is fixed in ice-cold 96-100% ethanol in approximately 1 ml of each sample. After at least 12 hour of fixation the sample was again centrifuge, excessive ethanol was removed by twice wash with phosphate buffer **(Vindelov, 1977)**. 200 µl of cell suspension in citrate buffer were transferred in a 5 ml tube. The solution of propidium iodide (0.05µl/ml) was protected against light with tinfoil during preparation, storage and the staining procedure. The solutions were mixed and the sample was filtered (12x75mm, cat. no.2058, falcon comp). The samples were run in the flow cytometr (Becton Dickinson, Sunnyvale, CA, USA) within 1 hour after the addition of propidium iodide and the stained samples were stored over night in room temperature to measure the sub G1 peak for apoptosis % in the same tubes according to **Cohen and Al-Rubea (1995)**. The DNA fluorescent histogram derived from flow cytometry was obtained with a computer program for Dean and Jett mathematical analysis **(Dean and Jett, 1974).**

**Histopathological examination**

Specimens which collected from liver and gills were fixed in neutral buffer formalin and were processed for histopathological investigation using light microscope according to **Bancroft and Stevens (1996)**. Another samples which fixed by immersion in 3% glutaraldehyde solution for 2 hrs followed by post-fixation in 1% osmium tetraoxide ($O_2O_4$) in 0.1 M phosphate buffer (pH 7.3) for 2 hrs at 4°C. Then the tissues were dehydrated in up-graded ethanol and finally embedded in. Araldite 502 resin. Semi-thin sections (1µm) were stained with double stained with uranium acetate and lead citrate and photographed with a transmission electron microscopy (TEM).

**Statistical analysis**

Data of the current study was statistically analyzed using the computer program **SPSS/PC (2001)**. The statistical method was one way ANOVA test.

## 3. Results

### The effect on MDA, GSH concentration and the antioxidant enzymes activity

Table 1 demonstrated that Nile catfish exposed to 1/20 $LC_{50}$ of $AlCl_3$ for six months showed significant increase ($P \square 0.05$) in the concentration of MDA in liver and gills comparing with the corresponding control groups, meanwhile this increase was mitigated in the group exposed to combined doses of $AlCl_3$ and Vit C. Moreover, the obtained data in Table 1 recorded an obvious significant decrease ($P \square 0.05$) in the activity levels of SOD and CAT and GSH concentration in liver and gills of fish exposed to $AlCl_3$ which alleviated in that group exposed to the combined doses comparing with the control group.

**Table (1):** The effect on MDA, GSH concentration and the antioxidant enzymes activity in liver and gills of Nile catfish exposed to $AlCl_3$ (1/20 $LC_{50}$), Vit C (5 ppm) and the combination for six months comparing with control group (Mean ±SE) (n=12).

| Parameters / Organs and Groups | MDA (nmole/g. tissue) | GSH (mg/g. Tissue) | SOD (U/g. tissue) | CAT (µmole $H_2O_2$ decomposed /g. tissue) |
|---|---|---|---|---|
| **Liver** | | | | |
| **$AlCl_3$** | 87.04±1.40 [a] | 114.60±3.52 [d] | 53.40±1.20 [c] | 23.48±0.70 [c] |
| **$AlCl_3$+Vit C** | 76.26±1.65[b] | 146.52±8.05 [c] | 64.60±1.56[b] | 34.68±2.26[b] |
| **Vit C** | 55.58±0.40[d] | 260.06±17.9[a] | 76.40±2.97 [a] | 68.80±4.57 [a] |
| **Control** | 71.36±1.19 [c] | 198.56±4.66[b] | 66.00±1.41[b] | 32.74±3.25[b] |
| **Gills** | | | | |
| **$AlCl_3$** | 44.20±1.53 [a] | 42.82±3.69[d] | 22.00±2.94 [c] | 9.64±0.92 [c] |
| **$AlCl_3$+Vit C** | 36.04±0.86 [b] | 58.22±1.15[c] | 34.80±1.56 [b] | 14.80±0.28 [b] |
| **Vit C** | 18.20±2.08[c] | 83.22±1.79 [a] | 41.60±1.07[a] | 17.72±0.45[a] |
| **Control** | 35.00±1.37 [b] | 70.26±1.63 [b] | 35.60±1.63 [b] | 14.90±0.44[d] |

Means within the same column carrying different superscripts are significant at $p \square 0.05$

### The effect on the level of DNA damage

As determined in the present study; the alkaline comet assay has adequate sensitivity for assessing the levels of primary DNA damage in liver and gills of both treated and control groups as depicted in Table 2 and Figures 1&2; which indicated that $AlCl_3$ exposed group recorded significant increase ($P \square 0.05$) in value of all parameters in both liver and gills (tail length, tail moment and DNA%) when comparing with corresponding control group. However the fish exposed to the combined doses of $AlCl_3$ and Vit C, ameliorated the level of primary DNA damage which illustrated via significant decrease ($P \square 0.05$) of all comet parameters compared to control groups of both liver and gills.

**Table (2):** The effect on oxidative DNA damage level (tail length, DNA% and tail moment) and apoptotic cell population (apoptosis %) in liver and gills of Nile catfish exposed to $AlCl_3$ (1/20 $LC_{50}$), Vit C (5 ppm) and the combination for six months comparing with control group (Mean ±SE) (n=12).

| Parameters / Organs and Groups | Tail length (µm) | DNA (%) | Tail moment (Units) | Apoptosis % |
|---|---|---|---|---|
| **Liver** | | | | |
| **$AlCl_3$** | 9.46±0.23 [a] | 10.18±0.56 [a] | 91.24±0.63[a] | 78.45±6.32[a] |
| **$AlCl_3$+Vit C** | 7.61±0.58[b] | 7.27±1.122[b] | 70.89±3.92[b] | 63.92±3.92[a] |
| **Vit C** | 2.56±0.098 [c] | 2.74±0.117 [c] | 7.51±0.432 [c] | 26.60±1.69[c] |
| **Control** | 2.53±0.094[c] | 2.81±0.02 [c] | 7.81±0.26[c] | 27.77±2.20[c] |
| **Gills** | | | | |
| **$AlCl_3$** | 9.20±0.164 [a] | 10.31±0.427 [a] | 92.23±3.21 [a] | 63.92±3.92[a] |
| **$AlCl_3$+Vit C** | 7.85±0.044[b] | 8.44±0.566[b] | 69.49±3.44[b] | 45.72±1.002[a] |
| **Vit C** | 2.54±0.043 [c] | 2.59±0.040[c] | 6.61±0.083 [c] | 25.02±1.29[c] |
| **Control** | 2.75±0.047 [c] | 2.80±0.023[c] | 7.32±0.319 [c] | 24.47±2.11[c] |

Means within the same column carrying different superscripts are significant at $p \square 0.05$

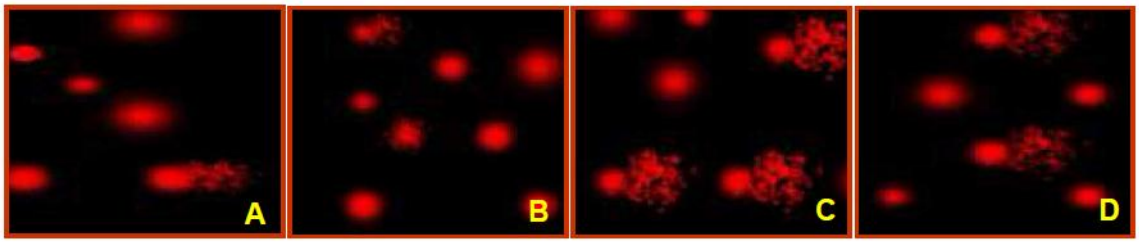


**Figure (1):** Comet images of cells derived from liver of Nile catfish of control group (A), Vit C (5 ppm) exposed group (B), $AlCl_3$ (1/20 $LC_{50}$) exposed group (C) and combined doses of $AlCl_3$ and Vit C exposed group (D) for six months.

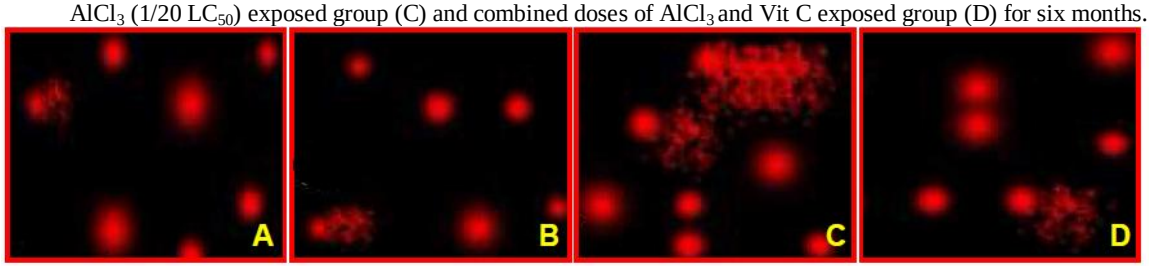


**Figure (2):** Comet images of cells derived from gills of Nile catfish of control group (A), Vit C (5 ppm) exposed group (B), $AlCl_3$ (1/20 $LC_{50}$) exposed group (C) and combined doses of $AlCl_3$ and Vit C exposed group (D) for six months.

**The effect on the population of apoptotic cells**

The results of apoptotic cell population are presented in Table 2 and Figure 3 which revealed pronounced significant induction ($P$□0.05) in the percentages of apoptotic cells in liver and gills of $AlCl_3$ exposed group comparing with the control groups. Meanwhile, the frequency of apoptotic cells was significantly declined ($P$□0.05) in both liver and gills of catfish exposed to the combined doses of $AlCl_3$ and Vit C when comparing with corresponding control groups.

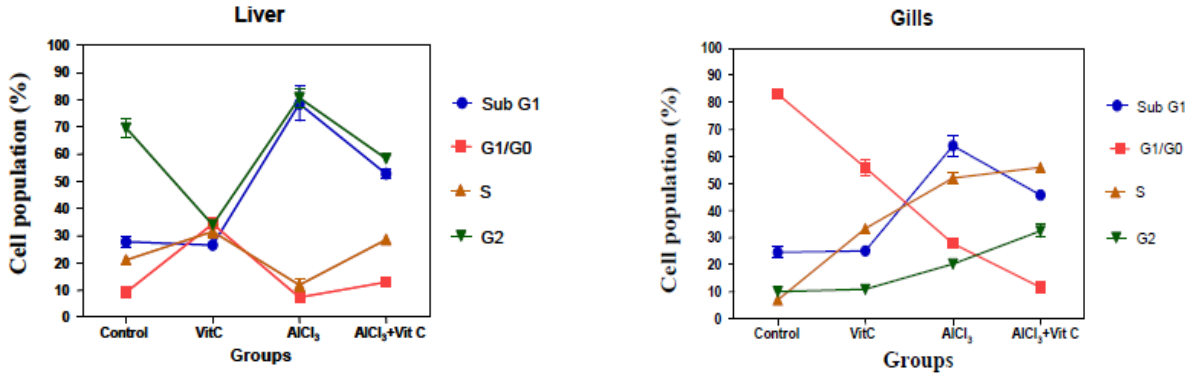


**Figure (3):** The effect on the apoptotic cell population (Sub G1 %) by flow cytometric analysis in liver and gills of Nile catfish exposed to $AlCl_3$ (1/20 $LC_{50}$), Vit C (5 ppm) and the combination for six months comparing with control group (Mean ±SE) (n=12).

**Histopathological results**

Light microscopical examination of liver and gills of control and Vit C groups showed normal histopathological structure **(Figures 4A, 5A)**. In group exposed to 1/20 $LC_{50}$ of $AlCl_3$; The liver revealed multifocal areas of coagulative necrosis which represented by pyknosis and karyolysis. Some of these areas were focally replaced by lymphocytes and macrophages and others displaced by melanomacrophages (**Figure 4B**). Diffuse hepatocyte degenerations of vacuolar and hydropic types and fatty change were seen besides, activation of melanomacrophages centers. The hydropic degeneration was accompanied with severe congestion of the hepatic sinusoids and the central veins (**Figure 4C**). The epithelial lining of the bile ducts showed vacuolar degeneration and others revealed hyperplasia in the lining epithelia. The pancreatic acini were necrotic and infiltrated with lymphocytes. The gills of the same group showed telangiectasia of the branchial blood capillaries, hemorrhages and lymphocytes aggregations particularly at the base of the primary lamellae. Focal patches of coagulative necrosis in the gill-filaments were observed with lymphocytic aggregations at the gill-tips (**Figure 5B**). Focal fusions of the secondary lamellae were manifested by hyperplasia of its epithelial lining and obliteration of interlamellar spaces. There was also an increase in activity and number of the goblet cells. Lamellar disorganization and desquamation or sloughing of its epithelia was focally detected.

While, in the group exposed to the combined doses of $AlCl_3$ and Vit C. The liver showed mild focal hydropic degeneration in the hepatocytes and sinusoidal congestion (**Figure 4D**). The hepatocytes showed vacuolated cytoplasm and rarely individual cell necrosis (**Figure 4E**). The portal areas were infiltrated with few lymphocytes. Few extravasated erythrocytes were rarely seen among the degenerated hepatic cells. The gills of that group showed mild congestion and hyperplasia of the respiratory epithelium with basal fusion of the secondary lamellae (**Figure 5C**). Few lymphocytes together with a considerable number of EGC were detected at the base of gill filaments.

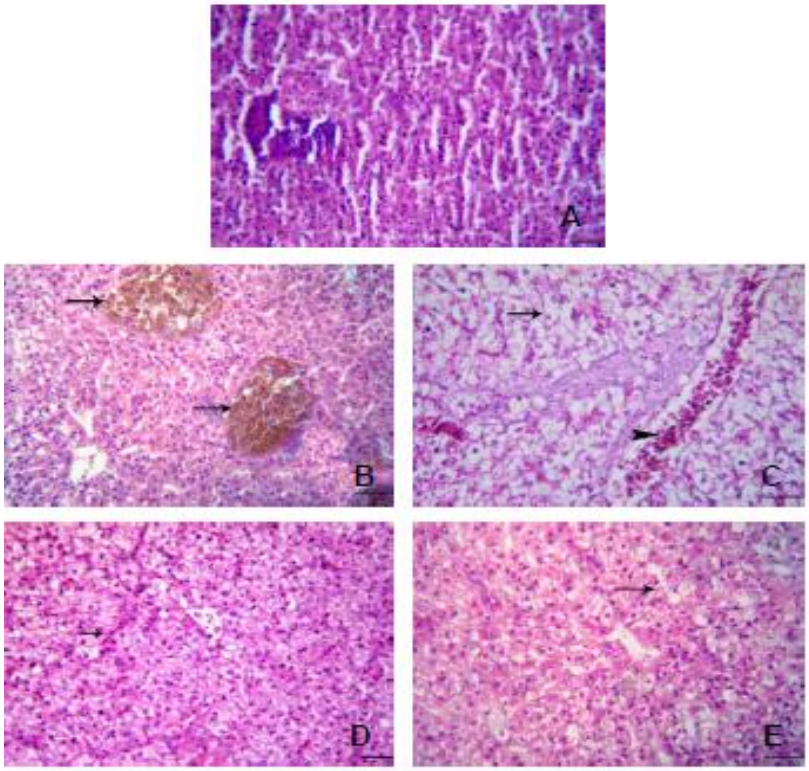


**Figure (4): A;** Section of control Nile catfish liver showing normal hepatocyte and sinusoidal architecture, HE (Bar = 100 µm).
**B** and **C;** Section of Nile catfish liver of $AlCl_3$ (1/20 $LC_{50}$) exposed group for six months showing, **B;** necrotic areas replaced by numerous melanomacrophages (arrows), **C;** diffuse hepatocytes degenerations (arrow) and severe congestion (arrowhead), HE (Bar = 100 µm).
**D** and **E;** Section of Nile catfish liver of the group exposed to combined doses of $AlCl_3$ and Vit C (5 ppm) for six months showing, **D;** mild hydropic degeneration and sinusoidal congestion (arrow), **E;** focal vacuolations in the hepatocytes (arrow), HE (Bar = 100 µm).

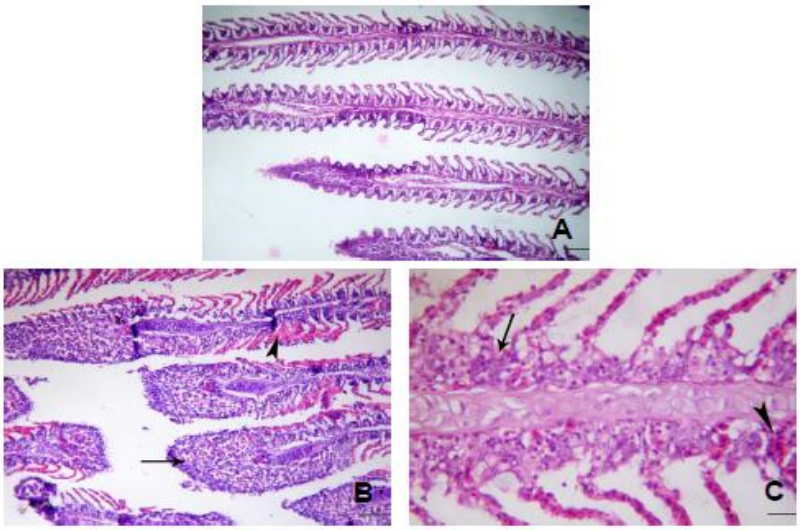


**Figure (5): A;** Section of control Nile catfish gills showing normal filaments and lining epithelium, HE (Bar = 100 µm).
**B;** Section of Nile catfish gills of $AlCl_3$ (1/20 $LC_{50}$) exposed group for six months showing focal patches of coagulative necrosis in the lining epithelia of secondary lamellae (arrowhead) and extensive round cells aggregations on the the tips of primary lamellae (arrows), HE (Bar = 100 µm).
**C;** Section of Nile catfish gills of the group exposed to combined doses of $AlCl_3$ and Vit C (5 ppm) for six months showing mild congestion (arrowhead) and hyperplasia of the respiratory epithelium with basal fusion of the secondary lamellae (arrow), HE (Bar = 100 µm).

Ultrastructural findings of liver cells of control and Vit C exposed group were identical with that generally known. The cytoplasm showed numerous mitochondria, rough endoplasmic reticulum, Golgi apparatus, glycogen granules and few fat lipid globules. The nucleus was normal round with centrally located nucleolus and dispersed granular chromatin. Moreover, the Vit C exposed group showed an increase of the leukocytes in the blood sinusoids and proliferation of bile canaliculi with large aggregates of RER (**Figure 6**). The hepatocytes of the group exposed to aluminum chloride showed severe vacuolation and lysis of the cytoplasm (rarefaction) with complete disappearance of the mitochondria, Golgi apparatus and glycogen. Narrowing or complete obliteration of the spaces among the hepatocytes were visualized with thinning of the cytoplasmic microvilli. Few rough endoplasmic reticulums were aggregated and condensed around the nuclei with partial lysis of its membranes and detached ribosomes. The nucleus showed marked crenation (indentation) and vacuolar detaching of nuclear membranes, dislocation of nucleolus, clumping and condensation of chromatin in the shape of an irregular homogenous ring of a snowflake form with peripheral migration into the cytoplasm. Partial lysis of the outer and intactness of the inner nuclear membrane was also observed with appearance of irregular shaped nucleus (polypoid). Fragmentation of the nuclear chromatin was detected and dispersed in the cytoplasm with absence of the nuclei inside the hepatocytes. Numerous inflammatory cells of macrophages and neutrophils containing numerous lysosomes were seen among the degenerated or necrotic hepatocytes (**Figures 7 and 8A**).

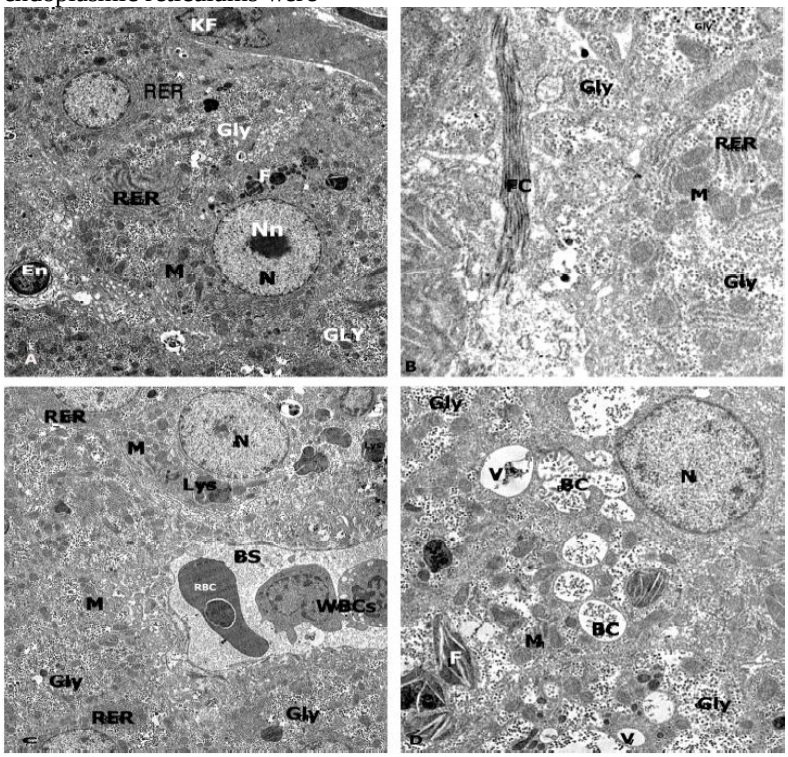


**Figure (6): A** and **B;** The hepatocytes of control. (N=nucleus, Nn= nucleolus, KF=kupffer cell, En= endothelium, M=mitochondria, RER=rough endoplasmic reticulum, FC=Golgi apparatus, Gly=glycogen, F=lipid globule, Lys=lysosome). **C** and **D;** Vit C exposed group, the liver showing increase the numbers of leukocytes in the blood sinusoid (WBC) and proliferation of bile canaliculi (BC) between the hepatocytes. TEM x 5850.

The hepatocytes of group exposed to the combined doses of $AlCl_3$ and Vit C showed mild to moderate vacuolations of the cytoplasm with few swollen mitochondria with amorphous deposits in the matrix and dilated regular shaped RER. Depletion or complete absence of glycogen and increase lipid globules in the cytoplasm was detected. Activated cytosome (peroxisome) and proliferation of bile canaliculi were also detected. The nucleus was nearly normal with mild clumping of chromatin. The inflammatory cells of predominate neutrophils were noticed (**Figures 8 B,C,D**).

The gills of control and Vit C exposed groups were normal with gill filaments, lamellae and surface epithelium. Meanwhile the gills of aluminum chloride exposed group showed severe vacuolation and necrosis pavement (PC) and epithelial cells (EPC) with dilated mucous cells (MC). These cells were separated from secondary lamella by edema containing fibrin and inflammatory cells. The previous findings were lowered or absent with restored the lining epithelium in group exposed to the combined doses of $AlCl_3$ and Vit C; Mild activation of the mucous and chloride cells (CC) were detected **(Figure 9)**

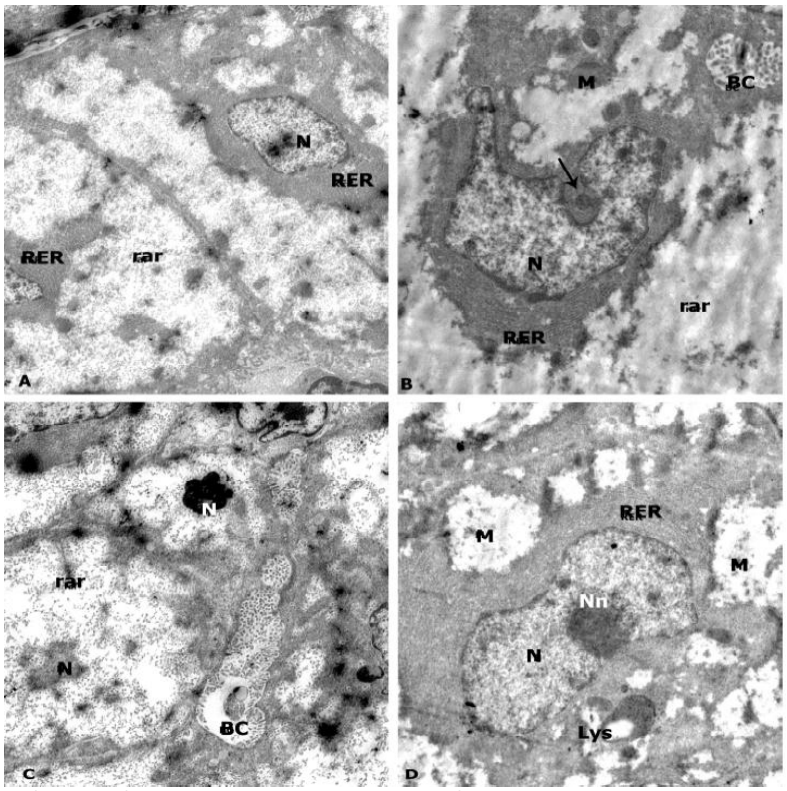


**Figure (7):** Section in liver of Nile catfish exposed to $AlCl_3$ (1/20 $LC_{50}$) for six months; **A;** The hepatocytes vacuolation and lysis of the cytoplasm (rar) with complete disappearance of the mitochondria, Golgi apparatus and glycogen. **B;** Marked crenation (indentation) and vacuolar detaching of nuclear membranes. **C;** Clumping and condensation of chromatin in the shape of polypoid mass. **D;** Macrophages among the hepatocytes (N=nucleus, RER=rough endoplasmic reticulum, BC=bile canaliculi). TEM x 5850.

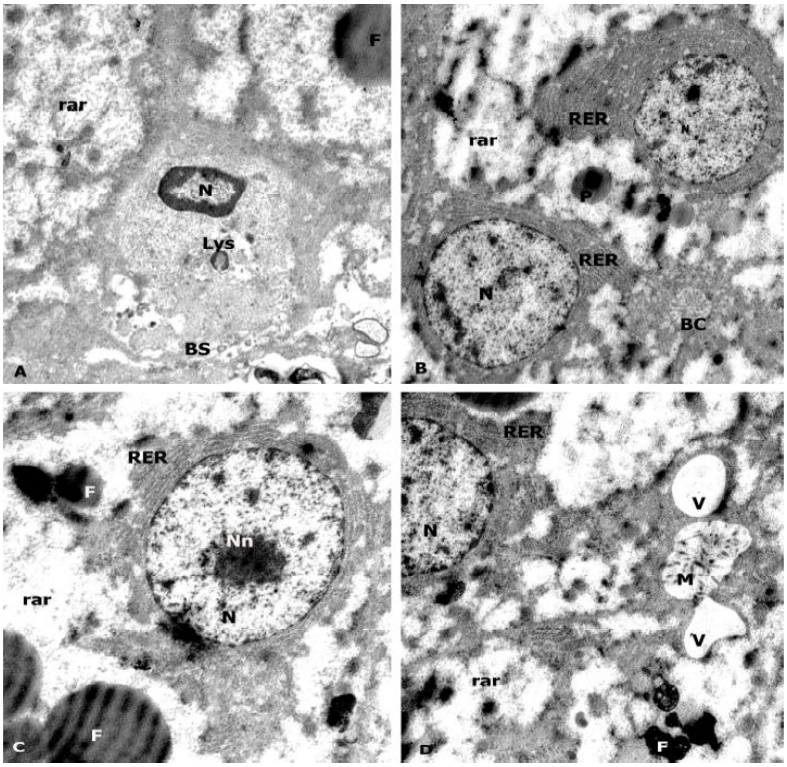


**Figure (8):** **A;** Section in liver of Nile catfish exposed to $AlCl_3$ (1/20 $LC_{50}$) for six months showing neutrophil containing numerous lysosomes (Lys) inside the blood sinusoid. **B, C** and **D;** Section in liver of Nile catfish exposed to combined doses of $AlCl_3$ and Vit C (5 ppm) for six months showing **B;** Moderate vacuolations of the cytoplasm (rar) with few swollen mitochondria, dilated regular shaped RER and peroxisome. **C;** Normal nucleus (N) and nucleolus (Nn) with deposition of lipid globules (F). **D;** Swollen mitochondria with amorphous deposits (M), vacuoles, lipid globules (F) and rarefaction (rar). TEM x 5850.

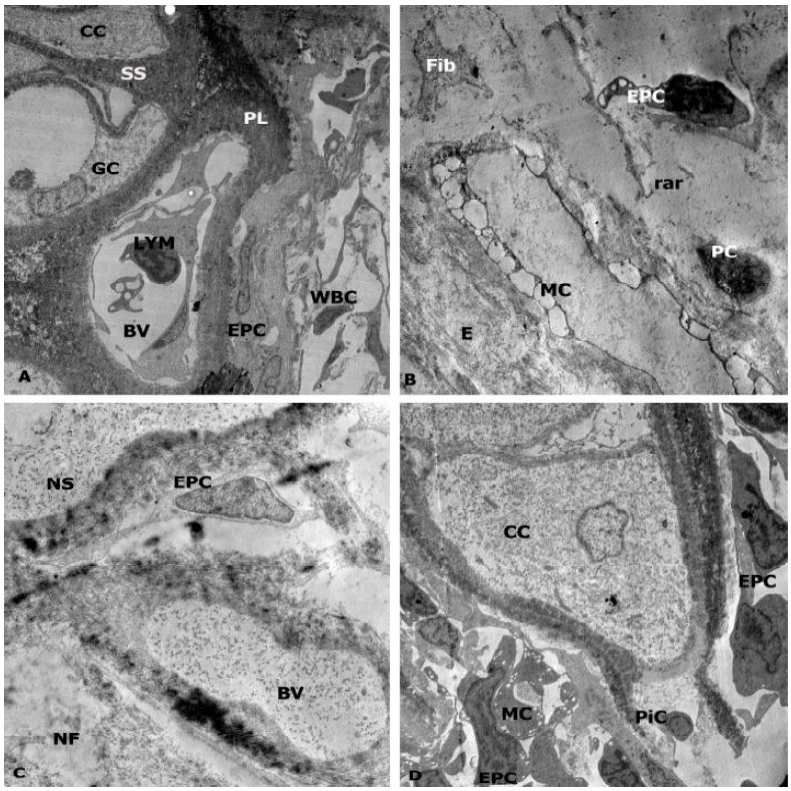


**Figure (9): A;** Section in gills of control Nile catfish showing normal primary (PL) and secondary (SS) lamellae, epithelial lining (EPC), chloride (CC), pavement (PC), goblet (GC) and pillar (PiC) cells besides normal blood vessels (BV). **B** and **C;** Section in gills of Nile catfish exposed to $AlCl_3$ (1/20 $LC_{50}$) for six months showing moderate vacuolations of the cytoplasm (rar) with edema (E) containing fibrin (Fib), dilated mucous cells (MC) and necrotic pavement (PC) and epithelium (EPC). **D;** Section in gills of Nile catfish exposed to combined doses of $AlCl_3$ and Vit C (5 ppm) for six months showing slight activation of mucous (MC) and chloride (CC) cells with normal other lining epithelia. TEM x 5850.

## 4. Discussion

The pollution of the aquatic environment with metals has become a serious health concern because of their toxicity and accumulation by organisms **(Mendil *et al.*, 2010 and Shah *et al.*, 2010)**. Due to its ubiquity, environmental exposure to aluminum may play an important role in the etiology of several diseases **(Migliore and Cappede, 2002)**. Fish in comparison with invertebrates, are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health **(Moiseenko, et al., 2008)**. The pro-oxidant/ antioxidant balance and scavenging of ROS are essential in order to maintain cellular homeostasis **(Valavanidis *et al.*, 2006)**.

Concerning MDA which is a byproduct of lipid peroxidation, its concentration reflects the level of lipid peroxides (LPX) very well **(DeZwart *et al.*, 1999)**. The data of the present study revealed significant increase in mean values of MDA concentration in liver and gills of $AlCl_3$ exposed fish (Table 1). Meanwhile, SOD which is an endogenous enzymatic scavenger that can counterbalance the oxidative destruction of free radicals **(Ryan *et al.*, 2008)**, the effect of $AlCl_3$ on the level of the antioxidant enzymes activities including SOD and CAT and GSH concentration was mirrored in our study by significant decrease in all activities and concentration in both liver and gills (Table 1).

The results are in accordance with **Dua and Gill (2001)** who observed a significant decrease in the activities of SOD and CAT in cerebrum, cerebellar hemisphere and brain stem after AL exposure. In alike manner those findings previously reported by **Yousef (2004)** and **Newairy *et al.* (2009)** who mentioned that that Al-induced changes in biochemical parameters, increased lipid peroxidation and decreased the activities of the antioxidant enzymes in plasma and different tissues of male rabbits and rats. **Yousef and Salama, (2009)** mentioned that $AlCl_3$ caused significant decrease in the activities of CAT and glutathione S-transferase (GST) and GSH concentration in male rats. Similarly, **Raina *et al.* (2010)** depicted that exposure to Aluminum phosphide at the concentration of 10 mg/kg/day results in enhancement of 20% in the levels of lipid peroxidation. Also, **Ding and Yang (2010)** found that $AlCl_3$ induced increase of LPX in the cells which was reflected by a reduction of SOD activity.

One possible alternative scenario which speculate the suppressive effect of aluminum on the antioxidant enzymes activities and its inducible effect on MDA reported by **Xie and Yokel (1996)** who indicated that the mechanism of Al-induced

toxicity may attributed to the potentiation of $Fe^{2+}$ to $Fe^{3+}$ to cause oxidative damage. Also, we could attribute our results to that opinion reported by **Kong *et al.* (1992)** and **Exely (2004)** who suggested a number of mechanisms had been proposed the ability of aluminum to increase the susceptibility of membrane lipids to peroxidative damage by producing rearrangement in cellular fluidity through binding to lipids in phospholipids membranes, others had proposed the ability of aluminum to catalyze superoxide radical anion-mediated biological oxidation.

Another explanation; Aluminum may have altered the cellular redox state by inhibiting the enzyme involved in antioxidant defense (SOD and CAT ) which function as blockers of free radical processes as postulated by **Nehru and Anand (2005)**. Moreover, it can be hypothesized the oxidative stress during aluminum exposure was attributed to electron leakage, enhanced mitochondrial activity and increased electron chain activity. ROS subsequently attack almost all cell components including membrane lipids and producing lipid peroxidation **(Flora *et al.*, 2003 and Turner and Lysiak, 2008).**

In the opposite direction, **Bhalla and Dhawan (2009)** found that administration of $AlCl_3$ (100 mg/kg b.wt/day/orally)in rats significantly increased the enzyme activities of CAT, SOD and glutathione reductase (GR) which speculated by the explanation of **Goncalves *et al.* (2008)** who point out that this increased SOD activity may be related to a potential adaptation mechanism of the organisms aimed at offsetting Al-induced oxidative stress. Also, **Bagnyukova *et al.* (2006)** state LPX products appear to be involved in the up-regulation of some antioxidant enzymes while diverse studies have shown that exposure to Al induces the expression of certain genes, including some which encode the synthesis of antioxidant enzymes **(Simonovicova *et al.*, 2004)**. The variations from our results may be attributed to the difference in species, dose and duration of exposure.

The aforementioned picture of the present study regarding the oxidative stress came in harmony and confirmed by our histopathological findings (Figures 4-9) including coagulative necrosis, pyknosis, karyolysis, hydropic and vacuolar degeneration, rarefaction, indentation, fragmentation of nuclear chromatin and vacuolar detaching of nuclear membranes.

In this respect, our results tie in that recorded by **Gonzalez *et al.* (2007)** and **Tripathi *et al.* (2008)**, they stated as a matter of fact, interactions between oxidative stress and hepatic damage may accelerate the progression of chronic hepatodegenerative disorders, including enzymes increase induced by aluminum. Also, in this direction our results are nearly similar with those obtained by **Wilhelm *et al.* (1996)** and **El-Demerdash *et al.* (2004)** who postulated that the hepatic tissue damage reflects the direct toxic effect of aluminum.

Concerning to alleviating effect of vitamin C, the level of both MDA concentration and antioxidant enzyme activity in the group exposed to the combined doses of $AlCl_3$ and Vit C in the current experimental work could be supported by the attribution recorded by **Tsao (1997)** and **Huang *et al.* (2002)** where they mentioned that two major properties of Vitamin C make it an ideal antioxidant. First is the one-electron reduction potentials of both ascorbate and its one-electron oxidation product, the ascorbyl radical. These low reduction potentials enable ascorbate and the ascorbyl radical to react with and reduce basically all physiologically relevant radicals and oxidants. The second major property that makes Vit C such an effective antioxidant is the stability and low reactivity of the ascorbyl radical formed when ascorbate scavenges a reactive oxygen or nitrogen species.

With respect to aquatic organisms, information on the matter is scarce. Although a few reports have indicated that exposure to Al can affect their molecular and cellular structure and function, no previous studies on genotoxic and cytotoxic effects had been carried out in Nile catfish. In regard to the assessment of genotoxic effects provoked by exposure to $AlCl_3$ which was detectable by the alkaline comet assay; the present work depicted an obvious statistically significant increase in the number of DNA breaks in both liver and gills (Figures 1&2).

These results were compatible with those reported by **Lankoff *et al.* (2006)** and **Lima *et al.* (2007)** who recorded that aluminum- treated human cells indicated an increased level of DNA damage. Also, Our results coincide with the findings previously mentioned by **Mihaljević *et al.* (2009)** and **Ternjej *et al.* (2010)** where they found that aluminum induced DNA damage in *leech Hirudo verbena* hemocytes and erythrocytes of the mosquitofish *Gambusia holbrooki* respectively by measuring the comet tail length, tail intensity, and tail moment. Theoretically, Al could induce DNA damage via three mechanisms; modification of chromatin structure, induction of ROS, and liberation of DNase from the lysosomes. The first mechanism is supported by the finding that Al can influence the structure of chromatin **(Bharathi *et al.*, 2003)**. In addition many metals, possibly Al as well are known to inhibit DNA repair mechanism which would lead to DNA damage. The second mechanism is supported by the observations that treatment of cells with Al can lead to the formation of reactive oxygen species **(Anane and Creppy 2001)**, and the third mechanism is supported by the finding that Al can change the permeability of hepatic lysosomal membranes (**Vander Voet *et al.*,**

**1992)** and that is also inhibits the lysosomal proton pump **(Zatta *et al.*, 2002)**. Enhanced lysosomal membrane could lead to DNase being liberated into the cytoplasm and to its passage into nucleus, where it could cut DNA, it has been shown that DNase introduced into cytoplasm by electroporation is a potent inducer of cytogenetic damage **(Sayed Aly *et al.*, 2002)**. Another hypothesis could be explain our findings previously reported by **Alves de Almeida *et al.* (2007)** they mentioned that AL inducing DNA strand breaks via generating ROS which may alter nucleotide bases. The hydroxyl radical may remove hydrogen atoms from the sugar, particularly from carbon 4 of ribose, besides the reaction of the singlet oxygen with DNA gives rise to OH-8 oxoguanine, which may newly react with another singlet oxygen and produce O OH-8 oxoguanine, oxidation in the sugar or DNA bases can generate mutations and DNA damage.

On that contention our experiment revealed that the group exposed to the combined doses of $AlCl_3$ and Vit C recorded an obvious reduction in oxidative DNA damage, which may be supported by the opinion of **Carr and Frei (1999)** as they found that Vit C can protect indispensable molecules such as proteins, lipids and nucleic acids (DNA and RNA) from damage by free radicals and ROS that can be generated either during normal metabolism or through exposure to toxins and pollutants.

Our study imply a significant increase in the frequency of apoptotic cells in both liver and gills of Nile catfish exposed to 1/20 $LC_{50}$ of $AlCl_3$ (Table 2 & Figure 3) on the same context those findings previously recorded by **Griffioen *et al.* (2004)** they mentioned that incubation of Al with human neuronal cells (NT2) produced significant apoptosis even in lower doses. Similary the study of **Banasik *et al.* (2005)** demonstrated an increase in the number of apoptotic cells in cultured human lymphocytes. Besides, **Tuneva *et al.* (2006)** recorded that in acutely isolated cerebellar granular cells, Al has been found to cause a rapid neurotic cell death. One possible mechanism is that Al binds to certain functional groups in the gill epithelium, increasing its permeability to ions and accelerating cell death **(Exley *et al.*, 1991)**, another attribution is that Al enhance the production of cytochrome C, which trigger the cell death cascade process **(Griffioen *et al.*, 2004)**. Moreover, the explanation of **Verstraeten and Aimo (2008)** where they declared that Al is able to displace Fe and promote the fenton reaction as well as to directly damage mitochondrion affecting electron transport in the respiratory chain, consequently, this increase ROS production, disrupts the membrane potentional, decrease the ATP stock and leads to disturbances of the cell cycle and apoptosis. Also, in situations where the generation of free radicals exceeds the capacity of antioxidant, defense, oxidative stress may lead to cell membrane degradation, cellular dysfunction and apoptosis **(Lucca *et al.*, 2009)**. Besides, it could be supported by the findings of **Griffitt *et al.* (2011) where** they depicted that exposure to $AlCl_3$ resulted in down regulation of genes involved in cell cycle regulation and inhibition of apoptosis.

The aforementioned results of the present experiment concerning $AlCl_3$ inducing apoptosis could be confirmed by our histopathological manifestations previously recorded. Meanwhile, the group exposed to the combined doses of $AlCl_3$ and Vit C showed a pronounced reduction in the percentage of apoptotic cells. This picture supported by the postulation based on the findings of **Satoh *et al.* (2007)** who indicated that Vit C protect Al induced cell death by increasing the concentration of intracellular GSH as confirmed in our experimental work.

## Conclusion

The present study points out relevant results that $AlCl_3$ elucidated marked ruinous effects on the oxidative and genotoxic impacts as well as the histopathological alterations which were alleviated by Vitamin C.

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