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Case history.Acute toxicity of cadmium in quail and treatment with N-Acetyl cystine

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Abstract

Cadmium (Cd) is a hazardous environmental and industrial toxicant so, the primary objective of this study was to investigate the protective effects of NAC on a case history of cadmium toxicities in quail birds. Eighty quails in same weight and age were used in this study and classified into 4 groups 20 quail for each .Forty quail chicks were taken from a farm with pervious case history of cadmium toxicity in water, and forty apparently healthy quail chicks were obtained from a farm. G1 was control group. G2 (collected from toxicated farm) supplied with normal water intoxicated with cadmium chloride to reach the same dose of cadmium in the farm with case history of cadmium toxicity.
.G3 (collected from toxicated farm) supplied with normal water intoxicated with cadmium chloride to reach the same dose of cadmium in G2 and treated with N-acetylcysteine. G4 normal quail birds treated with NAC only. This study conducted for 3 weeks. Liver function tests (AST, ALT and total bilirubin), renal function test (serum creatinine and uric acid), determination of cadmium residues in serum and liver, growth hormone (GH) and cortisol hormone were measured in serum, tumor necrosis factor TNF-α was measured in liver in addition to histopathological changes in liver of quails were conducted. Our results showed that, cadmium significantly increased AST, ALT, bilirubin, creatinine, uric acid, Cortisol hormone level, TNF-α expression in liver and cadmium residues in serum and liver while significantly decreased serum albumin and growth hormone (GH). Biochemical data were well supported by histopathological, residual estimation and TNF findings. NAC supplementation have protective role in cadmium toxicity in quail chicks and improve different estimated parameters toward control values. This treatment with NAC exhibits potent antitumor responses result from an early inhibition of TNF-α expression .

Keywords: *Cadmium toxicity, n-acetylcysteine, quail chicken, TNF-α.*

Introduction

Cadmium (Cd) was found to be a major heavy metal utilized in different agricultural and industrial processes. However, exposure to this element was found to cause serious environmental pollution and induce deleterious effects on human and animal health **(Abd-El-Moneim *et al.*, 2018).**

This metal accumulates mostly in liver and kidney, although its toxicity can extend to other organs such as lung , bladder, brain , bone, and reproductive system. It causes deleterious effects and a variety of diseases such as cancer, genetoxicity, reproductive disorders and infertility, arteriosclerosis, immune toxicity, and anemia **(Skipper *et al.*, 2016).**

The toxicity of Cd might be due to its metabolites in the liver leading to the generation of reactive oxygen species (ROS) that include hydrogen peroxide and superoxide anion .ROS can attack cellular biomolecules in several organisms resulting in deleterious oxidation of protein, formation of lipid peroxidation, reduction of antioxidant parameters, DNA damage,

chromosomal aberrations, alterations of gene expression, and apoptosis **(Kaur and Sharm 2015**).
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It is also well known that Cd impacts the antioxidant enzyme system and inflammatory reaction. Cd accumulates in the liver and kidney and presents as a complex bound to the metal-binding protein metallothionein (MT). Cd-MT complex is a temporary detoxifying mechanism of Cd demonstrated by humans and other mammals, Cd toxicity induced the release of TNF-α and IL-6 in rats, which is associated with systemic oxidative stress and may be involved in the Cd toxicity mechanism, Oxidative stress and apoptosis were induced by Cd due to depletion of antioxidant enzymes. Exposure to Cd for 28 days induced hepatic toxicity in quail by measuring body weight and biochemical parameters. illustrated that the high messenger RNA (mRNA) expression of HSPs and inflammatory cytokines may play a role in the resistance of liver toxicity in ducks induced by Mo and Cd **(Cao *et al.,* 2015).**

An Endocrine Disrupting Chemical (EDC) is an exogenous substance that causes adverse effects on health in intact organisms and their progeny, secondary to changes in endocrine function **(European Environment Agency 1996).**

Growth hormone (GH) is the most abundant and perhaps one of the most important peptide hormones secreted by the hypophysis. This molecule, a 191-amino-acid polypeptide, is important not only in growth action but also in very wide fields, such as carbohydrate and lipid metabolism. GH has multiple actions on different target tissues, which include bone, cartilage, adipose tissue, muscle, the heart, and the immune system (**De Palo *et al.*, 2001).**

Domesticated Japanese quail (*Coturnix coturnix japonica*) is medium-sized fowl which more eaten by Egyptians due to its low cost, rapid growth, early onset of lay, high reproduction rates, and low feed intake. Japanese quail meat is high protein content (19.6%), low-fat content (12.1 mg/100 g meat), low calorific value (192 g kcal/100 g meat), the highest amount of omega 3 fatty acids and vitamin A (**Ahmed *et al.,* 2017).**

N-acetylcysteine (NAC) is an organosulfur antioxidant derived from Allium plants. It is reported to exert a hepato protective activity; NAC has both mucolytic and ant carcinogenic properties. As a source of sulfhydryl groups, NAC is able to restore endogenous antioxidant potential, promote detoxification and act as a strong scavenger of toxic radicals such as OH• and H2O2. NAC was also shown to have anti-inflammatory and immunomodulatory effects, leading to increase of liver repair. It is a standard chemo protective drug against the toxicity of carcinogenic metals. Additionally, NAC has cyto protective effect caused by inorganic arsenic **(Abu El-Saad *et al.,* 2016)**.

N-acetylcysteine (NAC), a small molecule containing a thiol group and a precursor of reduced glutathione (GSH), It acts as a protective agent of the liver and kidney due to its ant oxidative properties and as a chelating agent in the elimination of metals due to its thiol group (**Kaplan *et al.,* 2008**).

N-acetylcysteine (NAC) is a direct antioxidant and a GSH precursor; previous studies showed that NAC prevented Cd-induced oxidative stress and liver toxicity **(Guo *et al.,* 2018)**.

Jicang *et al.,* (2014) recorded that Cadmium (Cd) is a hazardous environmental and industrial toxicant that has been classified as a type I carcinogen. Cd can indirectly promote the generation of ROS such as superoxide anions, hydroxyl radicals, and hydrogen peroxide and considered NAC as agent has a beneficial role in protecting cells against Cd-induced toxicity and ROS production
The primary objective of this study was to investigate the protective effects of NAC on some biochemical parameters, growth hormone, cortisol hormones, residues of Cd in serum and liver, TNF-α and histopathological changes in liver of Cd intoxicated quail chicks.

Materials and Methods
Total eighty Japanese quails weighted (70-80 g), two weeks age , were used in this study.
Forty Japanese quail chicks were taken from a farm with pervious history of cadmium toxicity

and forty apparently healthy Japanese quail chicks were taken from a private farm. Water and ration samples were taken from both farms to measure the cadmium residues. Each water sample (100 ml) was collected using clean tube sampler and kept refrigerated on icebox. Furthermore, each feed sample (100 g) was collected and kept in clean polyethylene bags. All samples were transferred for analysis.

Twenty Japanese quails from non-toxicated quails were feed ration and normal water which cadmium residue within permissible limits for 3 weeks (G1) as control group.

Twenty quails (collected from toxicated farm) (G2) were supplied with normal water intoxicated with cadmium chloride to reach the same dose of cadmium in the farm with case history of cadmium toxicity .

Twenty Japanese chicks (collected from toxicated farm) were feed with ration mixed with N-acetyl cysteine (800mg/kg ration) and normal water intoxicated with cadmium chloride to reach the same dose of cadmium in the farm with case history of cadmium toxicity for 3 weeks to study the effect of NAC on cadmium toxicity in quails (G3).

Twenty Japanese quails were feed ration mixed with NAC (800mg/kg ration) and normal water for 3 weeks (G4).

Quail chicks were reared during the experimental period in conventional type cages and kept under the same managerial, hygienic and environmental conditions. Birds were exposed to 23h light: 1 h dark, were fed on a standard ration with chemical composition of Crude protein % 18,digestible energy Kcal/kg of diet 2600 ,crude fiber % 10-12, Ca% 1.2, Ph.% 0.8 lysine % 0.75, methionine and cysteine % 0.65. The drinking water was available ad libitum throughout the study. Drinkers and feeding troughs were daily cleaned. The birds' health status was monitored throughout the trial. The basal experimental diet was formulated to cover the nutrient requirements of growing Japanese quail chicks as recommended by **National Research Council (1994).** cadmium residue estimated in water 3.12 ppm and ration 0.54ppm table (3)

Sampling:
After three weeks blood samples were collected from quails (G1, G2, G3, and G4) into plain centrifuge tubes. Samples were centrifuged at 3500 rpm for 15 min and serum was separated and stored at −20°C for serum biochemical analysis. Samples of liver were obtained from slaughtered quail for cadmium residue analysis, tumor necrosis factor TNF-α expression and histopathological examination.

Serum biochemical analysis:
Serum biochemical parameters were determined: aspartate amino transferase (AST), alanine amino transferase (ALT) activities according to **Reitman and Frankle (1957),** total bilirubin **Watson and Rogers., (1961)** and albumin as described by **Dumas *et al.,* (1971**). Determination of uric acid level and serum creatinine carried out according to **Caraway, (1963) and Giorio, (1974),** respectively**.** Were estimated in serum using commercial biodiagnostic kits.

Cadmium residue:
Cadmium residue were estimated in water, ration , serum and liver collected from slaughtered birds by using Atomic Absorption Spectrophotometer model SensAA Australia according to **(AOAC, 1990)**

Growth hormone and Cortisol hormone:
Serum concentration of GH and cortisol hormone was assayed by ELISA technique and ELISA kit (ELISA kit, Cusabio, China) according to the manufacturer's instruction.

Expression Tumor necrosis factor (TNF-α) in liver :
RNA extraction. RNA extraction from tissue samples was applied using QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH) where 30 mg of the tissue sample was added to 600 µl RLT buffer containing 10 µl β-mercaptoethanol per 1 ml. For homogenization of samples, tubes were placed into the adaptor sets, which are fixed into the clamps of the Qiagen tissue Lyser. Disruption was performed in 2 minutes high-speed (30 Hz) shaking step. One volume of 70% ethanol was added to the

cleared lysate, and the steps were completed according to the Purification of Total RNA from Animal Tissues protocol of the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH).
N.B. On column DNase digestion was done to remove residual DNA.

B. Oligonucleotide Primers. Primers used were supplied from **Metabion (Germany)** are listed in table (2).

C. Taqman rt-PCR. PCR amplifications were performed in a final volume of 25 µl containing 3 µl of RNA template, 12.5 µl of 2x QuantiTect Probe RT-PCR Master Mix, 8.125 µl PCR grade water, 0.5 µl of each primer of 20 pmol concentration and 0.125 µl of each probe (30 pmol conc.) and 0.25 µl of QuantiTect RT Mix. The reaction was performed in a Stratagene MX3005P real time PCR machine.

D. Analysis of rt-PCR results. Amplification curves and ct values were determined by the stratagene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the CT of each sample was compared with that of the positive control group according to the "ΔΔCt" method stated by **Yuan *et al*., (2006)** using the following ratio: (2^{-DDct}).

Histopathological examination:
For histopathological examination, the collected specimens were fixed in 10% neutral buffered formalin for at least 24 hours and then routinely processed by conventional method and finally stained by Heamatoxylene and Eosin **(Suvarna et al., 2012).**

Statistical analysis:
Data were expressed as the mean ± standard error of the mean (SEM) for each group. Differences between groups were analyzed using one-way analysis of variance (ANOVA). P value of <0.05 was considered to be statistically significant **SPSS 14 (2006)**.

Result and Discussion
Serum biochemical results:
Our results are shown in Table (1)
Our results showed that toxicated birds group revealed significant increase in the activities of serum AST, ALT, and bilirubin level compared with that in control group, These result agreed with the findings of **(Srinivasan & Ramprasath 2011)**. While NAC supplementation improves the values of these enzymes activity and decreases their activities in treated group, but still higher than normal.

AST enzyme is found mainly in liver and heart cells. Therefore, any minor damage, inflammation and necrosis of liver cells release the enzyme then increase its level in serum. The ALT is transaminase enzyme mainly found in the cytoplasm of liver cells and to lesser extent in kidney, heart, skeletal muscle, plasma, and pancreas **Ebrahimi A. (2011)**. Increasing activity of this enzyme plays an important role in the use of amino acids in the oxidation process or glycogenesis. It can be considered a useful clinical index to detect damages in liver. In addition, heart failure, muscular dystrophy, bile duct obstruction, hemolytic anemia increases level of this enzyme in plasma **(Rao *et al.,* 2006)**.

Regarding to the results in table (1) serum albumin levels were significantly decreased in toxicated birds and non-significantly decreased in treated group **Dilek *et al.,* (2016)**. revealed that NAC protects the liver cells from the damage caused by CCl4. NAC can enter to the cells easily and it is used in vivo and in vitro studies as an antioxidant. It protects the liver cells by increasing GSH levels in cells (**Odewumi *et al.,* 2011)**. Similarly, results of the present study showed that NAC protect the liver cell from damage caused by Cd **(Fig 4).**

Hassan *et al.* (2012) reported a significant hypoalbuminema in Cd-administered rabbits**;** this decrease was attributed to the focal hemorrhages in liver parenchyma which ultimately resulted in decreased albumin genesis from hepatocytes. Hpoalbuminemia can be caused by various conditions, including nephrotic syndrome, hepatic cirrhosis, heart failure, and malnutrition; however, most cases of hypoalbuminemia are caused by acute and chronic inflammatory responses. Serum albumin level is an important prognostic indicator of liver function **(Uyanik *et al* 2001**).
Regarding to the effect of Cd on renal function

there was significant increases in serum creatinine and uric acid in toxicated chicks compared to control, while treated group show lowered levels than toxicated birds but still higher compared to control ones, this indicated that Cd leads to renal damage. **Abou-Kassem *et al.* (2016)** are in line with our results as they reported a dose-dependent increase in uric acid and serum creatinine levels in the quail fed with different levels of Cd polluted ration. The elevation in creatinine and uric acid level was due to the effect of Cd on renal tubules and glomeruli.

Cadmium residues:

The obtained results from samples collected from a farm with pervious history of cadmium toxicity in water, revealed that the mean residual concentration levels of cadmium in water samples for toxicated farm were 3.12 ppm and was 0.54ppm for feed ration samples.

The World Health Organization **WHO (2011)** set recommended limits to drinking water for livestock; these permissible limits were 0.05ppm for cadmium.

The Egyptian Organization for Standardization and Quality Control (**EOS, 2010)** set the permissible limit for cadmium in poultry meat which must be not exceeding than 0.05 mg/kg. The cadmium residues in both serum and liver samples, in toxicated birds showed highest values which are significantly increased compared to control. The average level of Cd in serum and liver of quail in NAC treated group recorded lowered values than toxicated group but still higher than control group. Cd residues in the serum and liver were decreased in the birds treated with NAC in diet than those unsupplemented group.

The recorded values are higher than the permissible limits. While adding NAC to ration decreases the residual values comparing to toxicated group but still high compared to control values. Tissue Cd concentrations in animals are closely related to Cd levels in feedstuffs and the duration of Cd load **(Nkansah and Ansah**, **2014)**. Nutritional and vitamin status, such as iron status, age and sex and a wide range of factors controlling absorption and accumulation of Cd in tis-sue (**T.H. Reem *et al.,* 2012)**. The obtained results came with agree with those obtained by **Herzig *et al.* (2007)** and **Youssef and Mansour, (2014)**, who recorded 0.3 mg Cd./kg in Egyptian poul-try meat recorded higher results of cadmium concen-tration levels in quail meat. Cadmium is poorly absorbed in the body, but as soon as absorbed and slowly excreted, like different metals, and accumulated. Cadmium is endo-crine demanding substance and may cause the devel-opment of prostate and breast cancers in addition to kidney and skeletal damage in humans, **(Saha and Zdwan, 2012)**.

N-acetylcysteine (NAC), is a small molecule containing thiol group and act as a precursor of reduced glutathione (GSH) it also has clinical usefulness in the treatment of acute heavy metal poisoning, its antioxidative properties and as a chelating agent in the elimination of metals due to its thiol group (**Odewumi *et al.,* 2011)**. Several reports have also indicated that GSH can form a complex with Cd and that it decreases the uptake of Cd into the cells and prevent toxicity of this metal **(Kimura *et al.,* 1997).**

In agreement with our results, **Odewumi *et al.,* (2011)**, found that Vit. C supplementation decrease Cd accumulation in liver of quail. On other hand **Herzig *et al.* (2007).** Reported that antioxidants supplementation did not decrease the concentration of Cd in the kidney.

Cortisol level

Regarding to serum cortisol level (table 1) cortisol level was significantly increased in toxicated birds as affected by Cd toxicity, compared to control. Treatment with NAC decreases cortisol level toward control values. Various studies are in agreement with our results, **Chowhdury *et al.,* (2004) and Wu *et al.* (2007)** have indicated that the primary response to Cd-induced stress is an indication of the elevation of serum cortisol level. The general adaptation response of the animal to stressors is the increase of cortisol secretion. It has been reported that animals living in contaminated environments for longer duration experiences periods of high metabolic activity that could eventually lead to impaired cortisol se-

cretion and cellular alterations. The direct toxic effect of Cd on adrenal tissue causes an elevated ACTH and hence, an increased serum cortisol level **(Yin *et al.* 2000). Laflamme *et al.*, (2000)** recorded that plasma cortisol levels were generally higher in sampled adult yellow perch from different lakes after cd exposure.

Growth hormone (GH)

Regarding to GH results recorded in table (1), toxicated group showed significantly decreased values and has the lowest vales compared to control group. Treated group recorded higher values than that of toxicated ones, but still lowered than control.

Earlier studies on assessment of growth hormone (GH) levels in Cd-administered rabbits have revealed that decrease in body weight is caused by alterations in the GH levels **(Lafuente *et al.*, 2000).** Cadmium exposure decreased the dopamine metabolism in all brain areas studied, and plasma levels of prolactin, GH, and ACTH were diminished. The Cd concentration did not increase in hypothalamus or in the pituitary after the metal exposure. These results suggest that Cd inhibits the secretion of these pituitary hormones and this inhibitory effect is not mediated by dopamine or the degree of metal accumulation (**Lafuente *et al.*, 2002**). As reported by **Lafuente *et al.* (2003)**, the effect of Cd on GH levels might vary according to the doses.

Exposure of developing rainbow trout to a nominal cadmium concentration resulted in a rapid accumulation of the metal from the surrounding water. This exposure resulted in a delay of GH transcription and translation until a later organogenic period compared with the control group. This interruption in GH expression may be due to somatotroph damage as previous authors have reported that cadmium exposure resulted in pituitary necrosis in a variety of vertebrate species **(Xiao *et al.*, 2018).**

Tumor necrosis factor (TNF -α) expression in liver

The results of TNF-α was shown in table (2), Fig. (1 & 2) toxicated birds showed the most higher level of TNF-α comparing to all other groups, while treating with NAC decreasing the values of TNF toward normal levels. Treated birds showed an improvement in TNF values but still higher than control.

The mechanisms of harmful effects of cadmium in cells include loss in anti-oxidant cabability decrease in thiol status, activation of signaling pathways, inhibition of DNA methylation and DNA repair, and cell damage **(Czeczot & Skrzy- cki, 2010).**

Cadmium is also thought to have carcinogenic characteristic the International Agency of Research on Cancer classified this metal and its compounds as Group 1 human carcinogens **(IARC, 2012).**

The crucial components of the inflammatory response are the vascular and cellular responses. Inflammation, either acute or chronic, is mediated by a multiple of chemical substances (proteins, lipids, lipoproteins) secreted by cells participating in the inflammatory process either directly and/or responding to the inflammatory stimulus **(Das, 2011).**

The Tumor-necrosis factor (TNF), a cytokine released by activated macrophages in response to infection, toxin and other harmful stimuli, is a necessary and sufficient mediator of local and systemic inflammation. TNF-α enhances and prolongs the inflammatory response by activating other cells to re- lease both cytokines such as IL-1, and mediators like eicosanoids, nitric oxide and reactive oxygen species, which promote further inflammation and tissue injury **(Tracey, 2002).**

The review of literature varied differential effects of cadmium exposure on TNF-α production and gene expression, depending on bio logical system studied (**Afolabi *et al.*, 2012; Cormet-Boyaka *et al.*, 2012**). However rather large concentrations of CdCl2 were needed to stimulate TNF-α secretion **(Dong *et al.*, 1998).** The scientist discovered a dose-response connection between Cd concentration and TNF-α production **(Haase *et al.*, 2010).** Cadmium exposure caused the release of TNF-α in THP-1 people monocytic leukemia cell line **(Freitas & Fernandes, 2011).** CdCl2 treatment (2.5 mg/kg b.w.) of ICR mice significantly increased the expression level of TNF-α mRNA in liver tissue as compared to control **(Lee &**

Lim, 2011). chronic treatment of Wistar rats to 15 ppm Cd generate a significant elevation in TNF-α values in cardiac tissue (Cd: 793 pg/g tissue, con- trol: 402 pg/g tissue) **(Yazihan *et al.,* 2011).** A treatment of a 7-week exposure of rats to 50 and 100 ppm cadmium by drinking water resulted in 336% and 470% increase in TNF-α plasma values, respectively, as compared to controls **(Afolabi *et al.*, 2012).**

Zhao *et al.,* (2006) investigated acute inflammatory response in the intestines of mice following CdCl2 oral exposure**.** The scientific discovered that both in mice orally treated with 25 mg/kg b.w. of CdCl2 and those with 100 mg/kg b.w. of CdCl2, the expression of TNF-α mRNA in duodenum and jejunum did not differ substantially from the control value from 0 to 24 hours with cadmium administration **.**

In the present study, all the birds in G4 (control birds treated with NAC only) behaved normally; their values for different parameters are near values of control group. Serum biochemical results, residue and histopathological results in these groups are normal as compared with the control group.

Since NAC is a thiol antioxidant precursor of glutathione (GSH), it reduced TNF-α and 4-HNE-protein adducts levels, inflammation, creatine kinase levels, and myonecrosis in diaphragm muscle **(De Senzi *et al.,* 2013).**

Victor *et al.* (2003) investigated the effects of NAC on the reducing condition of peritoneal macrophages and lymphocytes in a fatal endotoxic shock model experimentally created in rats. In the study, 150 mg/kg intraperitoneal NAC treatment administered 30 minutes after the lipopolysaccharide (LPS) procedure was found to reduce reactive oxygen species, TNF-α, MDA levels, and the oxidized reduced GSH rate. NAC was shown to prolong the survival of rats. NAC treatment suppressed the release of inflammation markers TNF-α, IL-6 and IL-10 in organ insufficiency related to endotoxin shock. It was claimed that liver, heart and kidney injuries were minimized with this useful effect of NAC **(Gul *et al.,* 2011).** NAC, has been shown to improve cardiac performance and liver functions via improving the hepatosplanchnic perfusion (**Del Sorbo and Zhang, 2004**).
On other hand, NAC induced a dose-dependent increase of membrane TNFa expression on 16-hour-stimulated T cells. This increase was significant from 4 to 96 hours **(Delneste *et al.,* 1997).**

Histopathological findings:
The liver Control of quail revealed normal arranged hepatic acini and surrounding sinusoids (Fig. 3A) In the cadmium treated groups the liver showed abundant abnormally divided hepatocytes (Fig.3B) and increase in mitotic division of the hepatocytes represented by more than one nuclei, the hepatocytes revealing pleomorphic anisokaryotic cells giving malignant hepatocellular carcinoma (HCC) (Fig. 3C) The control cadmium treated groups revealed abundant liver cells with trabecular pattern hepatocellular carcinoma (Fig3.D) that results were in agreement with **(Margeli, *et al.,* 1994, Elwakil *et al.,* 2017)** who reported that Cadmium toxicity related to the interaction with nucleic acid biosynthesis and the rate of DNA synthesis was suppressed markedly in the cadmium pretreated group and the first peak of liver regeneration was delayed. The rate-determining enzyme thymidine kinase was suppressed in the liver cadmium-treated groups also. The other microscopic lesions in Cd treated liver exhibited perivascular fibrosis and dilation of the portal vein (Fig. 4E) Multiple focal area of necrosis invaded by inflammatory cells were also observed (Fig.4F) that results were supported by **Elwakil *et al.,* (2017), Karina Martínez-Flores *et al.,* 1978)** who stated that exposure to environmental pollution of cadmium was associated with hepatic necroinflammation. Cadmium exposure causing hepatocyte swelling, fatty changes, focal necrosis, hepatocyte degeneration the damaged liver infiltrated by polymorphonuclear neutrophils (PMN), which, in addition to Kupffer cells contribute to the hepatotoxicity by enhancing inflammatory mediators and promoting necrosis that activated Kupffer cells release a number of inflammatory mediators that subsequently enhance the expression of adhesion molecules that initiate a cascade of cellular and humoral

responses leading to inflammation and secondary liver damage during Cd-induced hepatotoxicity it is known that activated Kuppffer cells, release a variety of cytotoxic mediators that can directly damage hepatocytes ,necrosis and vaccuolation of epithelial lining bile ducts and sloughing of necrotic cells into the lumen with severe vacuolation of the muscular medial layer of the portal artery were recorded in Cd treated groups (Fig. 4G) That supported by **Arroyo *et al.*, (2012**) who stated that liver is the main target organ of Cd toxicity following both acute and chronic exposure and the mechanisms of Cd toxicity in the liver. Included the disruption of the cellular antioxidant system and the decrease in thiol status, the generation of reactive oxygen species and oxidative stress, the interference of biological metal homeostasis, involvement of inflammatory mediators, disruption of cell adhesion and cell damage leading to cellular apoptosis. Severely dilated portal vein and coagulated red blood cells in the vein, newly formed bile ductules and portal fibrosis were also shown in the Cd treated groups (fig4.H)

Koçak and Akçil (2006) explored in cadmium exposed rats shortening in prothrombin time and activating partial thromboplastin time. Protein C and antithrombin decreased to statistically significantly lower levels in rat plasma after cadmium exposure, number of thrombocytes was also decreased. They concluded that chronic cadmium toxicity sets the stage for hypercoagulation and hence increases the risk of thrombosis. In the group treated by Cd +NAC lysis of the coagulated accumulated red blood cells and forming of tunnel was observed in the central vein (Fig5.I) Regeneration of the epithelial lining of the bile ducts which retain to normal histologic structure was seen in group treated by Cd and N acetyl cysteine as in (Fig.5J). That results were in agreement with **Khoshbaten *et al.*, (2010)** who stated that N-Acetylcysteine improves liver function in patients with Non-Alcoholic Fatty Liver Disease. N-Acetylcysteine (NAC) has a protective effect against liver injury.and recorded improvement of liver histopathology and reduction of oxidative stress by NAC They recorded a significant decrease in liver steatosis and fibrosis in patients with fatty liver NAC. They added that NAC have the ability to block the propagation of lipid peroxidation. Cd treatment+NAC groups exihibited hepatocytes with normal morphological histological structure except for small vaccuolation of the hepatocytes (fig5.K). Also revealed regression of the necrotic area and minimize of the inflammatory cells invaded (Fig.3K) that agreed with **M Z.E. Hala (2016) and El-Serafi *et al.*, (2018)** who recorded that hepatotoxicity showed significant atteneuation in the histological structure of liver treated with NAC and considered (NAC) as a glutathione precursor used in the treatment of acetaminophen hepatotoxicity

Groups treated with NAC only revealed heamolysis of red blood cells in portal vein (Fig.6L) that supported by **Jang *et al.*, (2013)** who recorded that N-acetylcysteine (NAC) artificially elevates prothrombin time (PT). All factors II, VII, IX, and X activity had a significant decrease with the addition of NAC. Also NAC treated groups showed filling of hepatocytes by vacuuolations making plumping of hepatocytes (Fig. 6M)

Conclusion

In our study Cd toxicity elected hepatotoxicity and nephrotoxicity, this was proven by different serum biochemical tests and histopathological findings. High levels of cadmium residues were recorded in serum and liver. High level of TNF-α are recorded. Adding NAC improve all tested parameters finding towored normal levels. Our study proves the protective effect of NAC against cadmium toxicity.

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Table (1): The effect of N acetyl cysteine (NAC) in cadmium toxicated quail chicks on serum biochemical parameter (n=5)

Groups / Parameters	(G1) Control	(G2) Toxicated by cadmium	(G3) Cadmiun + NAC	(G4) NAC
ALT (u/l)	13.78±1.5^{d}	61.78 ±8.5^{a}	30± 5.8^{c}	16.38 ±2.1^{d}
AST (u/l)	18.6 ± 0.75^{d}	78.6±2.91^{a}	71.2±3.42^{a}	34.2± 2.27^{c}
T. bilirubin (mg/dl)	0.752 ±0.08^{c}	1.404 ± 0.04^{a}	0.98± 0.04^{b}	0.71 ± 0.06^{c}
Albumin (g/dl)	3.9 ±0.16^{a}	2.82±0.06^{c}	3.64 ±0.05^{b}	3.82±0.41^{a}
Creatinine (mg/dl)	0.68 ±0.07^{d}	1.77 ± 0.12^{a}	0.99 ± 0.03^{c}	0.645±0.08^{d}
Uric acid (mg/dl)	2.62 ±0.13^{d}	6.92±0.388^{a}	3.26±0.32^{cd}	2.9±0.09^{d}
Serum Cadmium residue (ppm)	0.01 ± 0.002^{d}	0.34 ±0.04^{a}	0.17 ±0.02^{c}	0.016±0.01^{d}
Liver Cadmium residue (ug/g)	0.09 ± 0.01^{e}	1.76 ± 0.30^{a}	0.45 ± 0.06^{c}	0.09±0.01^{e}
Cortisol hormone (ug/dl)	259.8 ± 14.82^{e}	608 ± 27.05^{a}	391 ± 10.54^{c}	291±17.43^{de}
GH (ng/ml)	3.34 ± 0.25^{ab}	1.06 ± 0.05^{e}	2.66 ±0.19^{c}	3.67±0.29^{a}

AST: Aspartate transaminase, ALT: Alanine transaminase, (TB) total bilirubin, Alb.: albumin, Uric acid, (GH) growth hormone. Means within each raw bearing common superscript do not differ significantly (P<0.05).

Table (2). The effect of NAC in cadmium toxicated quail chickens on TNF-α. Expression in liver.

Gp ID.	28S rRNA	Tnf alpha	
	CT	CT	Fold change
G1) Control	20.87 ± 0.04	22.19± 0.28	
(G2) Toxicated by cadmium	20.81 ± 0.33	19.10± 0.47	8.1492 ± 0.80^{a}
(G3) Cadmiun + NAC	20.23 ± 0.48	20.27 ± 0.41	2.434 ± 0.13^{c}
(G4) NAC	19.99± 0.5	21.40 ± 0.53	0.9417 ± 0.05^{d}

Table (3). Cadmium residue in water and ration administered to the quails

Group / Residue	(G1) Control	(G2) Toxicated by cadmium	(G3) Cadmiun + NAC	(G4) NAC
Cd residue in water ppm	0.182	3.12	3.12	0.182
Cd residue in ration ppm	0.54	0.54	0.54	0.54

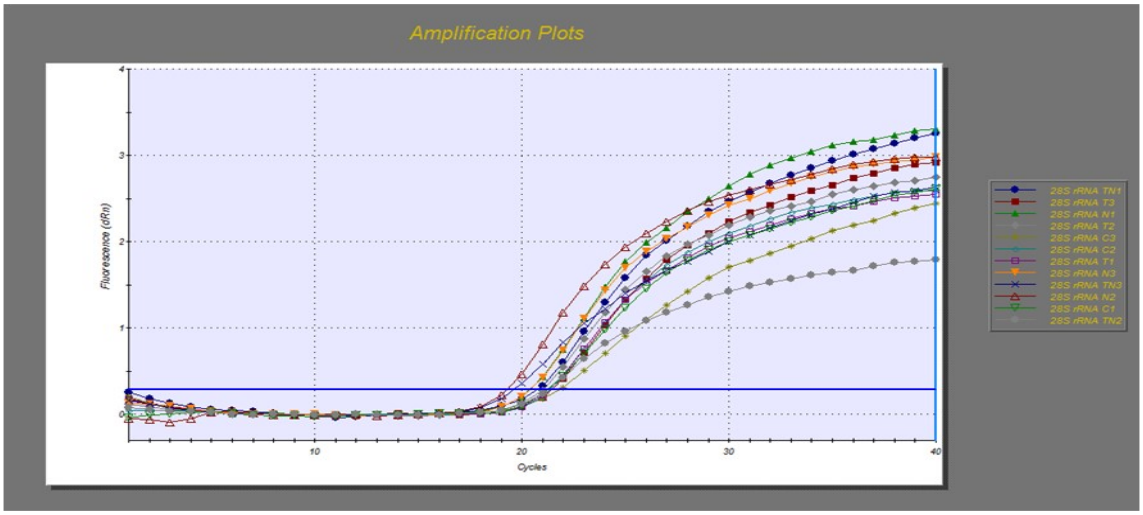

Fig. (1): The effect of NAC in cadmium toxicated quail chickens on 28S rRNA

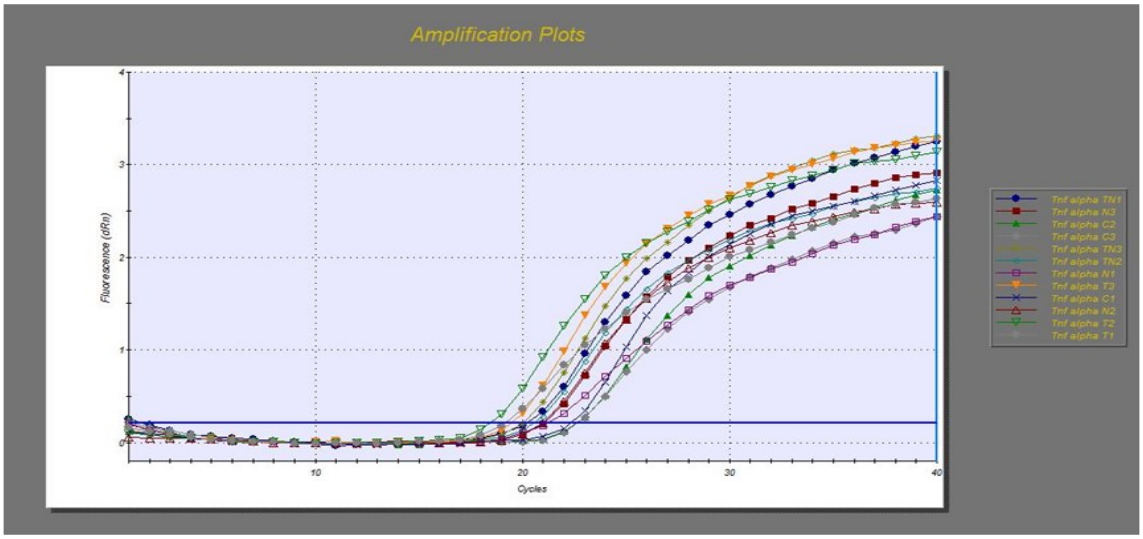

Fig. (2): The effect of NAC as protective agent in cadmium toxicated quail chickens on TNF-α expression in liver

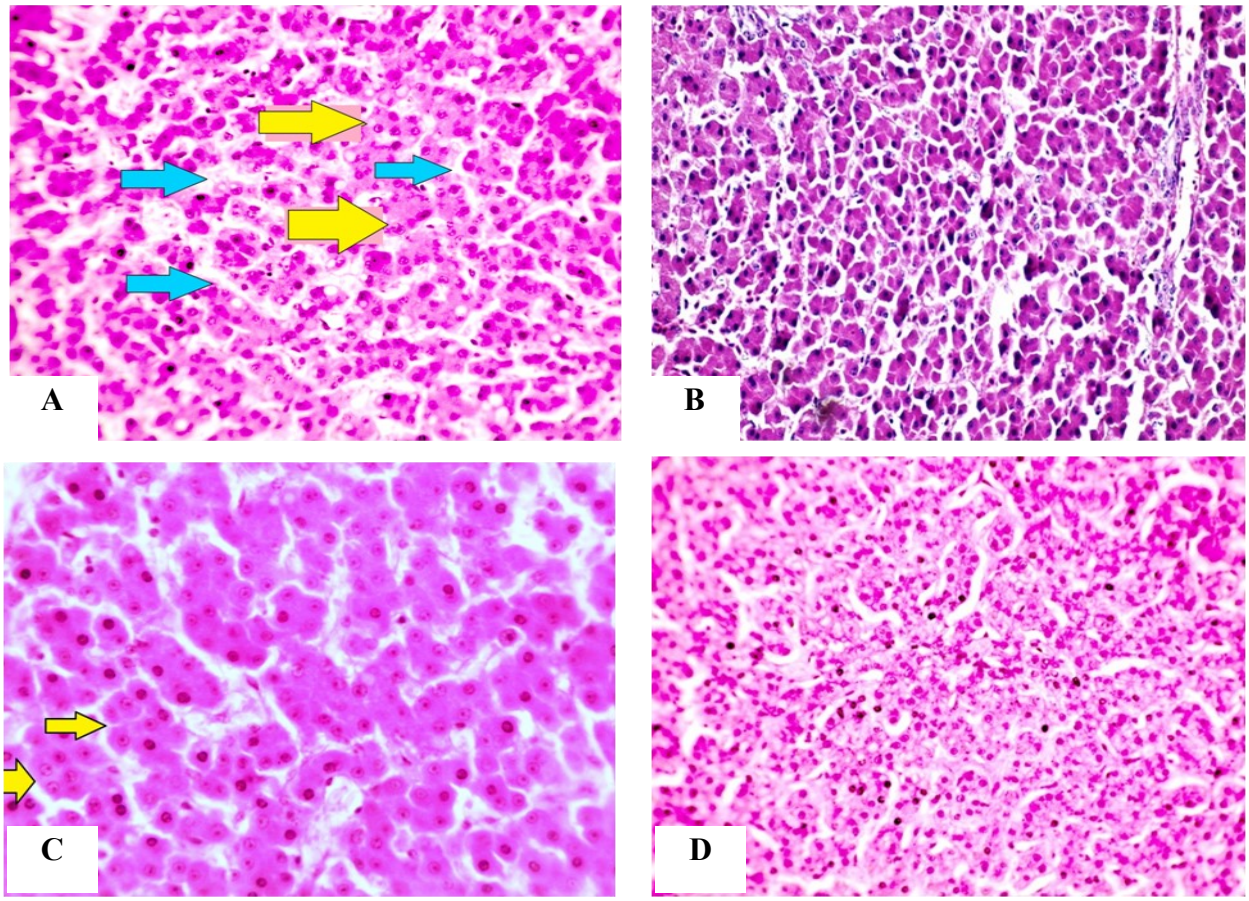

Fig. (3): **(A)** control nomral arranged hepatic acini (yellow arrows) and surrounding sinusoids (blue arrows) (StainH&EX400), **(B)** Liver quail intoxicated by cd showing abundant abnormally divided hepatocytes the hepatocytes revealing pleomorphic anisokaryotic cells giving malignant hepatocellular carcinoma (StainH&EX400) **(C)** High power of previous picture showing increase mitotic division of the hepatocytes represented by hepatocytes with more than one nuclei (yellow arrow), the hepatocytes revealing pleomorphic anisokaryotic hepatocytes giving malignant hepatocarcinoma (StainH&EX600) **(D)** Cd Controle showing abundant liver cells with trabecular pattern hepatocellular carcinoma with fatty vaccuolations hepatocellular carcinoma (StainH&EX200).

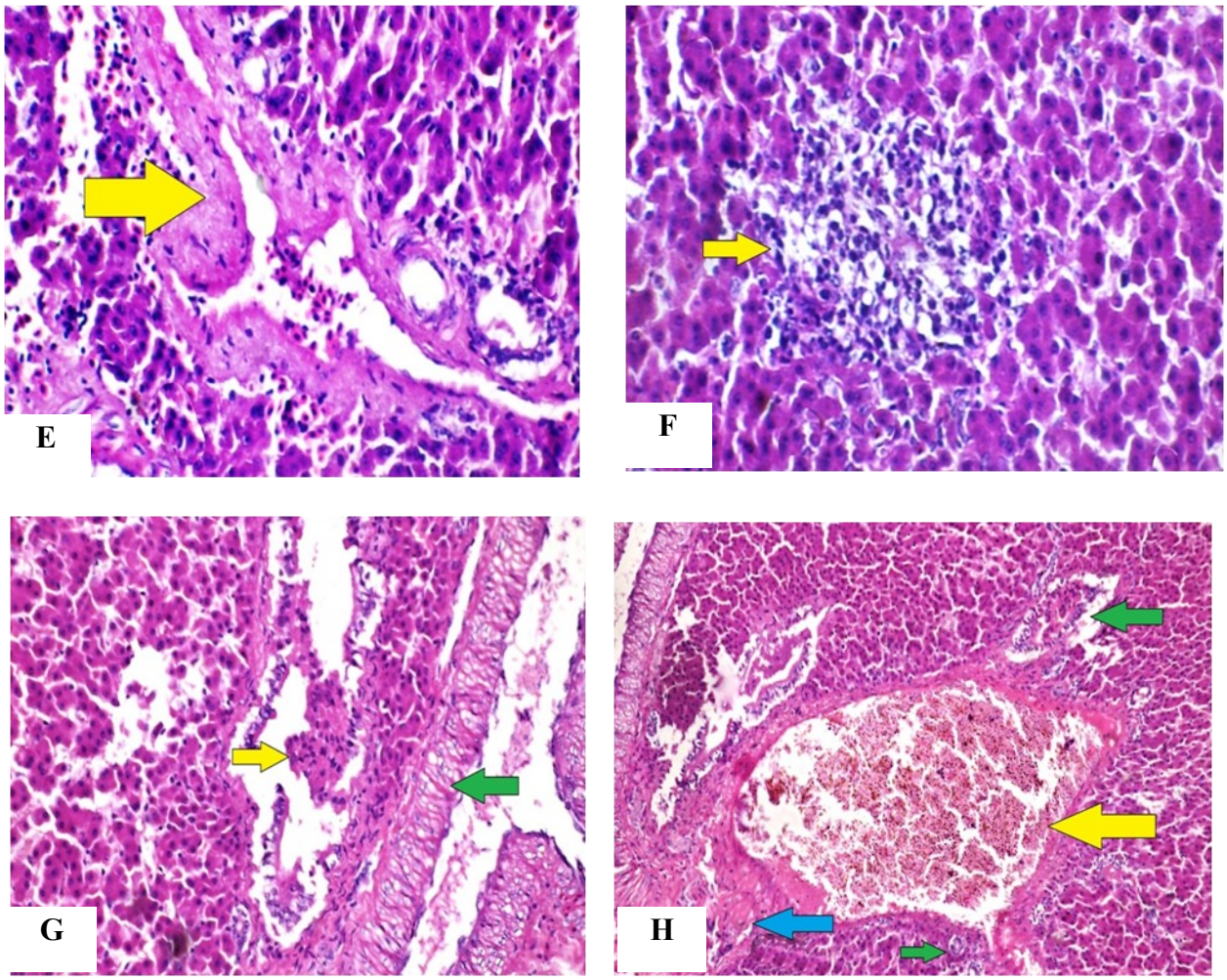

Fig. (4): (E) (Cd intoxicated liver showing perivascular fibrosis (yellow arrow) and dilation of the portal vein (Stain H&EX600) **(F)** Cd intoxicated showing multiple focal area of hepatocellular necrosis invaded by inflammatory cells (Stain H&EX400) **(G)** Cd intoxicated showing necrosis and vaccuolation of epithelial lining bile ducts and sloughing of necrotic cells into the lumen (yellow arrow) severe vacuolation of the muscular medial layer of the portal artery (green arrow) (Stain H&EX200) **(H)** Cd treatment showing severely dilated portal vein and coagulated red blood cells in the vein (thrombus) (yellow arrow) ,newly formed bile ductules (green arrows), portal fibrosis (blue arrow) (Stain H&EX200)

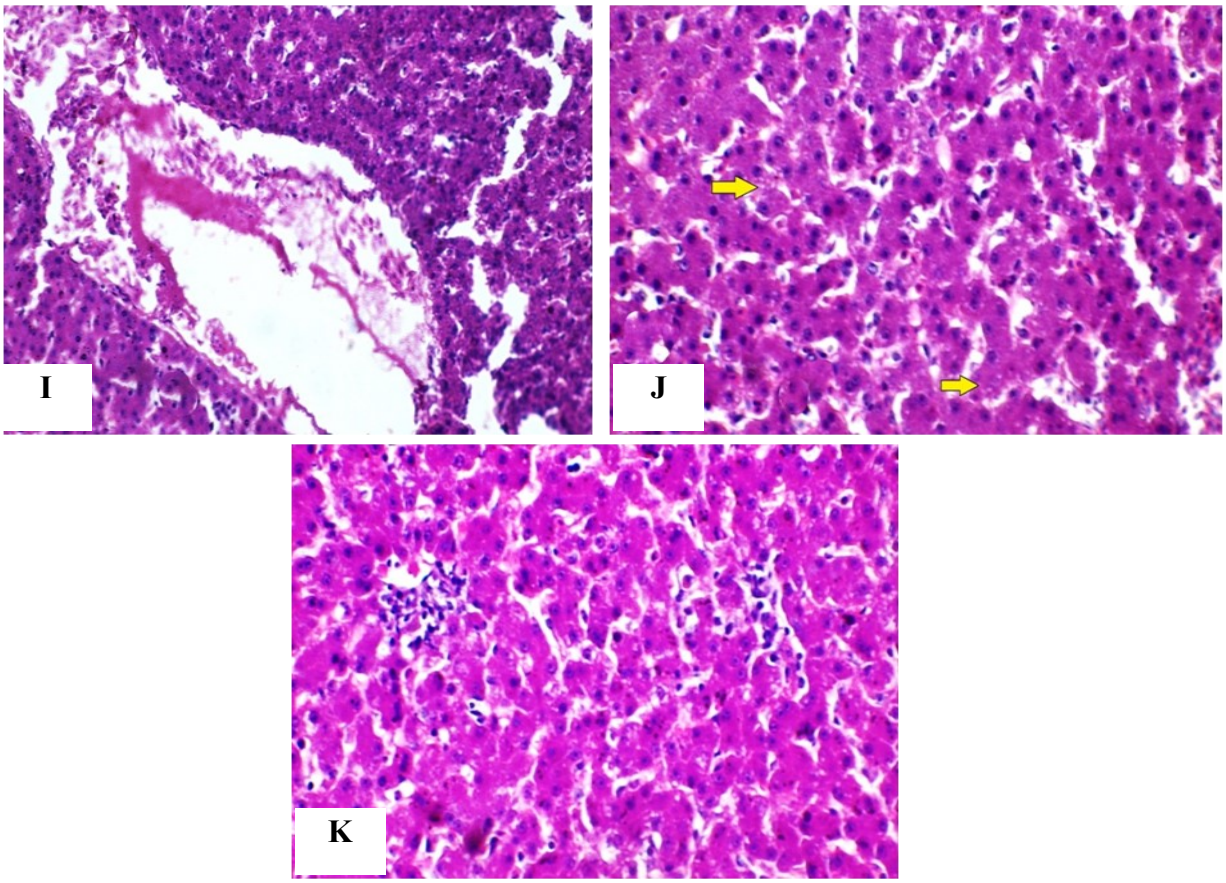

Fig. (5): (I) Cd intoxicated +NAC showing lysis of the coagulated accumulated red blood cells and forming of tunnels (Stain H&EX400 **(J)** Cd treatment +NAC retain the to normal histologic structure (yellow arrow) (Stain H&EX400) **(K)** Cd intoxicated+NAC showing normal hepatocytes with normal morphological histological structure except for small vaccuolation of the hepatocytes (Stain H&EX400

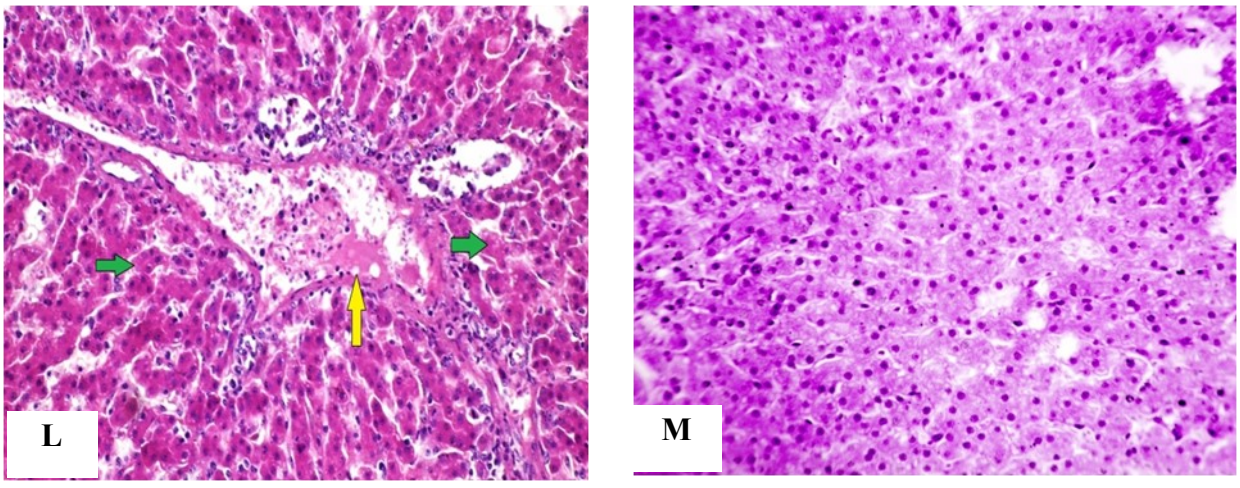

Fig. (6): (L) NAC Liver showing lysis of red blood cells (yellow arrow) in portal vein, vaccuolation of hepatocytes (green arrows) (Stain H&EX200) **(M)** NAC showing filling of hepatocytes by vacuolations making plumping of hepatocytes (Stain H&EX400)

References

Abd-El-Moneim, O.M.; Abd El-Kader, H.A.; Abd El-Rahim, A.H.; Radwan, H.A.; Fadel, M. and Farag, I.M. (2018). Modulatory role of *Saccharomyces cerevisiae* against cadmium-induced genotoxicity in mice. J Arab Soc Med Res; 12: 27-38.

Abou-Kassem, D.E.; Mahrose, K.M. and Alagawany, M. (2016). The role of vitamin E or clay in growing Japanese quail fed diets polluted by cadmium at various levels. Animal J. 10: 508–19.

Abu El-Saad, A.M.; Al-Kahtani, M.A. and Abdel-Moneim, A.M. (2016). N-acetylcysteine and meso-2,3-dimercaptosuccinic acid alleviate oxidative stress and hepatic dysfunction induced by sodium arsenite in male rats. Drug Design, Development and Therapy, 10, 3425–3434. http://doi.org/10.2147/DDDT.S115339.

Afolabi, O.K.; Oyewo, E.B.; Adekunle, A.S.; Adedosu, O.T. and Adedeji, A.L. (2012). Impaired lipid levels and inflammatory response in rats exposed to cadmium. EXCLI J 11: 677–687.

Ahmed, A.M.; Hamed, D.M. and El-sharawy, N.T. (2017). Evaluation of some heavy metals residues in batteries and deep litter rearing systems in Japanese quail meat and offal in Egypt, Veterinary World, 10(2): 262-269.

AOAC. (1990). Official Method of Analysis. 15th Ed., in Association of Official Analytical Chemists, Washington, DC, USA,1990.

Arroyo, V.S.; Karina Martínez Flores; Ortiz, L.B.; Enrique Gómez-Quiroz, L. and María Concepción Gutiérrez-Ruiz (2012). Liver and Cadmium Toxicity: Journal of Drug Metabolism & Toxicology 2157-7609 JDMT.

Caraway, W.T. (1963). Standard Methods of Clincal Chemistry. Edited by Seligsol, DAcademic Press, NewYork and London,4.

Cao, H.; Gao, F. and Xia, B. (2015). Alterations in trace element levels and mRNA expression of Hsps and inflammatory cytokines in livers of duck exposed to molybdenum or/ and cadmium. Ecotoxicology & Environmental Safety, 125: 93–101.

Chowhdury, M.J.; Mcdonald, D.G. and Wood, C.M. (2004). Gastrointestinal uptake and fate of cadmium in rainbow trout Acclimated to sublethal dietary cadmium. Aquat Toxicol **69**: 149-163, 2004.

Cormet-Boyaka, E.; Jolivette, K.; Bonnegarde-Bernard, A.; Rennolds, J.; Hassan, F.; Mehta, P.; Tridandapani, S.; Webster-Marketon, J. and Boyaka, P.N. (2012). An NF-κB–independent and Erk1/2-dependent mechanism controls CXCL8/IL-8 responses of airway epithelial cells to cadmium. Toxicol Sci 125: 418–429.

Crow, P.D.; Walter, B.N.; Mohler, K.M.; Otten-Evans, C.; Black, R.A. and Ware, C.F. (1995). A metalloprotease inhibitor blocks shedding of the 80-kD TNF-receptor and TNF processing in T lymphocytes. J Exp Med, 181: 1205, 1995.

Czeczot, H. and Skrzycki, M. (2010). Cadmium — element completely unnecessary for the organism. Postepy Hig Med Dosw 64: 38 –49.

Das, U.N. (2011). Inflammation. In: Molecular Basis of Health and Disease. Das UN, ed, pp 15–100. Springer Science+ Business, Media BV.

Delneste, Y.; Jeannin, P.; Potier, L.; Romero, P. and Bonnefloy, J.Y. (1997). *N*-Acetyl-L-cysteine exhibits antitumoral activity by increasing tumor necrosis factor α-dependent T-cell cytotoxicity. Blood 90 1124 –1132.

Del Sorbo, L. and Zhang, H. (2004). Is there a place for N-acetylcysteine in the treatment of septic shock? Crit Care; 8: 93-5.

De Palo, E.F.; Bassanello, M. and Lancerin, F. (2001). GH/IGF system, cirrhosis and liver transplantation, Clin. Chim. Acta 310(1), 31–37.\

De Senzi, M.; Pinto, R.; Ferretti, R.; Moraes, L.H.; Neto, H.S.; Marques, M.J. and Minatel, E. (2013). N-Acetylcysteine treatment reduces TNF-alpha levels and myonecrosis in diaphragm muscle of mdx mice. Clin Nutr, in press.

Dillek, A.; Yasemin, A.; Hasan, A.; Hatibe, K.; Aysegul, B. and Kamil, S. (2016). Protective effects of n-acetyl cysteine on oligosaccharide residues in the experimental liver intoxication formed by carbontetrachloride (CCl4) in rats Acta Medica Mediterranea, 32: 255.

Dong, W.; Simeonova, P.; Gallucci, R.; Matheson, J.; Flood, L.; Wang, S.; Hubbs, A. and Luster, M.I. (1998). Toxic metals stimulate inflammatory cytokines in hepatocytes through oxidative stress mechanisms. Toxicol Appl Pharmacol 151: 359–366.

Dumas, B.T.; Watson, W.A. and Briggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green .Clinical chemistry Acta 31, 87-96.

Ebrahimi, A. (2011). the clinical interpretation of medical tests. Publishing institute teymurzadeh. 2th edition. Tehran; 2011: 628. [Persian]

El-Serafi, I.; Remberger, M.; El-Serafi, A.; Benkessou, Zheng; Marte, E.; Per Ljungman, Mattsson, J. and Hassan, M. (2018). "The effect of N-acetyl-l-cysteine (NAC) on liver toxicity and clinical outcome after hematopoietic stem cell transplantation Sci Rep. 2018; 8: 829

Elwakil, M.; Mohamed, A.; Abo-Hashem, E.M.; Shabana, Y.M.; El-Metwally, M.A.; El-Kannishy, G.; El-Adl, A.M.; Anwar, R.; Fawzy, E.; Saied, N. and Elzayat, M.M. (2017). Hepatocellular Carcinoma in Populations Consuming Heavy Metals Contaminated Plants in North Delta, Egypt" Research Journal of Environmental Toxicology, 31, 78-81.

EOS (2010). Egyptian Organization for Standardization and Quality Control. Maximum Level for Certain Contaminants in Food Stuffs. ES No. 7136/2010.

EUROPEAN ENVIRONMENT AGENCY (1996). European workshop on the impact of endocrine disruptors on human health and wildlife, 2-4 December 1996, Weybridge, UK. Report of the Proceedings. Report EU 17549. Copenhagen: European Commission DG VII.

Freitas, M. and Fernandes, E. (2011). Zinc, cadmium and nickel increase the activation of NF-κB and the release of cytokines from THP-1 monocytic cells. Metallomics 3: 1238 –1243.

Giorio, J.D. (1974). Clinical chemistry-principle sand techniques Henry et.,ed., Harper and Row. Hagerstown. Pp. 543.

Gul, M.; Ayan, M.; Seydanoglu, A.; Cander, B.; Girisgin, S. and Erayman, I. (2011). The effect of N-acetyl cysteine on serum glutathione, TNF-alpha and tissue malondialdehyde levels in the treatment of sepsis. Ulusal travma ve acil cerrahi dergisi = Turkish journal of trauma & emergency surgery: TJTES. 2011; 17(4): 293–7. Epub 2011/09/22. Pmid: 21935824.

Guo, M.Y.; Wang, H.; Chen, Y.H.; Xia, M.Z.; Zhang, C. and Xu, D.X. (2018). N-acetylcysteine alleviates cadmium-induced placental endoplasmic reticulum stress and fetal growth restriction in mice. PLoS ONE 13(1): e0191667. https://doi.org/10.1371/journal. pone.0191667.

Haase, H.; Ober-Blöbaum, J.L.; Engelhardt, G.; Hebel, S. and Rink, L. (2010). Cadmium ions induce monocytic production of tumor necrosis factor-alpha by inhibiting mitogen activated protein kinase dephosphorylation. Toxicol Lett 198: 152–158.

Hassan, R.A.; Amin, D.M.; Rahmy, N.A.; Hatem, M.E. and Dessouky, M.I. (2012). Clinicopathological, histopathological and immunological studies on animals exposed to lead and cadmium under experimental conditions. New York Sci J **5**: 120-136, 2012.

Herzig, I.; Narratilora, M.; Suchy, P.; Vecerek, V. and Totusek, J. (2007). Model trial investigating retention in selected tissues using broiler chicken fed cadmium and humic acid. Vet. Med., 52(4): 162-168.

IARC (2012). International Agency for Research on Cancer, Cadmium and cadmium compounds. In: Arsenic, metals, fibres and dusts. A review of human carcinogens. IARC Monographs 100C, pp 121–145. IARC, Lyon, France.

Jang, D.H.; Weaver, M.D. and Pizon, A.F. (2013). "In vitro study of N-acetylcysteine on coagulation factors in plasma samples from healthy subjects".J Med Toxicol. 2013 Mar; 9(1): 49-53.

Jicang, W.; Huali, Z.; Xuezhong, L. and Zongping, L. (2014). "N-acetylcysteine protects against cadmium-induced oxidative stress in rat hepatocytes"J Vet Sci. 2014 Dec; 15(4): 485–493.

Kaplan, M.; Atakan, I.H.; Aydogdu, N.; Aktoz, T.; Ozpuyan, F.; Seren, G.; Tokuç, B. and Inci, O. (2008). Influence of N-acetylcysteine on renal and hepatic toxicity of cadmium in rats. Pediatr Nephrol. 2008; 23:233–241.[PubMed: 18064495].

Karina Martínez-Flores Sato, N.; Kamada, T.; Suematsu, T.; Shichiri, M.; Hayashi, N.; Matsumura, T.; Kishida, Y.; Abe, H.; Furuyama, F.; Ishibashi, F. and Hagihara, B. (1978). "Cadmium toxicity and liver mitochondria. I. Different effects of cadmium administered in vivo toadult, young, andethionine-fedrats" J Biochem. 1978; 84(1): 117-25

Kaur, S. and Sharma, S. (2015). Evaluation of toxic effect of cadmium on sperm count, sperm motility and sperm abnormality in albino mice. Int J Adv Res 2015; 3: 335–343.

Khoshbaten, V.; Manouchehr, B.; Akbar, A.; Koorosh, M.; Mohammad, K.; Sara, F.; Hosain, B.; Javad, K.; Maryam, Z.; and Farzad, N. (2010). N-Acetylcysteine Improves Liver Function in Patients with Non-Alcoholic Fatty Liver Disease Hepat Mon. 2010 10(1): 12–16.

Kimura, O.; Endo, T. and Sakata, M. (1997). Uptake of Cd-metallothionein into LLC-PK1 cells: A comparative study with CdCl2. Biol Pharm Bull 20: 158-162, 1997.

Koçak, M. and Akçil, E. (2006). The effects of chronic cadmium toxicity on the hemostatic system. Pathophysiol Haemost Thromb. 2006; 35(6): 411-6.

Lafuente, A.; Marquez, N.; Perez-Lorenzo, M.; Pazo, D. and Esquifino, A.I. (2000). Pubertal and postpubertal cadmium exposure differentially affects the hypothalamic-pituitary-testicular axis function in the rat. Food Chem Toxicol **38**: 913-923, 2000.

Lafuente, A.; Gonzalez-Carraced, A. and Marquez, N. (2002). Oral cadmium exposure throughout puberty does not inhibit secretion of prolactin, GH and ACTH through dopamine metabolism changes in male rat, J. Trace Elements Med. Biol. 16(4), 249–254 (2002).

Lafuente, A.; Cano, P. and Esquifino, A. (2003). Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? Biometals 16(2), 243 –250 (2003).

Laflamme, J.S.; Couillard, Y.; Campbell, P.G.C. and Hontela, A. (2000). Interrenal metallothionein and cortisol secretion in relation to Cd, Cu, and Zn exposure in yellow perch, Perca flavescens, from Abitibi lakes. Can. J. Fish. Aquat. Sci. 57, 1692–1700.

Lee, J. and Lim, K. (2011). Inhibitory effect of plant-originated glycoprotein (27 kDa) on expression of matrix metalloproteinase-9 in cadmium chloride-induced BNL CL.2 cells. J Trace Elem Med Biol 25: 239–246.

Margeli, A.; Theocharis, S.; Skaltsas, S.; Skopelitou, A. and Mykoniatis, M. (1994). 'Effect of cadmium on liver regeneration after partial hepatectomy in rats" Environ Health Perspect. Sep; 102 (Suppl 3): 273–276.

M.Z.E Hala (2016). The effect of N-acetylcysteine on the liver following aluminium chloride exposure in adult male albino rat: a histological and immunohistochemical study The Egyptian Journal of Histology ,39 (2) 150–161.

National Research Council (1994). Nutrient Requirements of Poultry: Ninth Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/2114.

Nkansah, A.M. and Ansah, J.K. (2014). Determination of Cd, Hg, As, Cr and Pb levels in meat from the Kumasi Central Abattoir. Int. J. Sci. Res. Publ., 4(8): 2250-3153.

Odewumi, C.O.; Badisa, V.L.D. and Le, U.T. (2011). "Protective effects of N-acetylcysteine against cadmium-induced damage in cultured rat normal liver cells," International Journal of Molecular Medicine, vol. 27, no. 2, pp. 243–248, 2011.

Reitman, S. and Frankel, S.E. (1957). Acolorimetric method for the determination of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase. American journal of clinical pathology 28, 56-63.

Rao, Y.V.; Das, B.K.; Jyotyrmayee, P. and Chakrabarti, R. (2006). Effect of Achyranthes aspera on the immunity and survival of *Labeo rohita* infected with Aeromonas hydrophila. Fish & shellfish Immunology 2006; 20(3): 263-73.

Saha, N. and Zaman, M.R. (2012). Evaluation of possible health risks of heavy metals by consumption of foodstuffs available in the central market of Rajshahi city, Bangladesh. Environ. Monit. Assess., 185: 3867-3878.

Skipper, A.; Sims, J.N.; Yedjou, C.G. and Tchounwou. P.B. (2016). Cadmium chloride induces DNA damage and apoptosis of human liver carcinoma cells via oxidative stress. International Journal of Environmental Research. Int. J Environ Res Public Health 2016; 13: 1–10.

SPSS, 14 (2006). Statistical Package for Social Science, SPSS for windows Release 14.0.0, 12 June, 2006. Standard Version, Copyright SPSS Inc., 1989-2006. SPSS Inc

Srinivasan, R. and Ramprasath, C. (2011). Protective role of silibinin in cadmium induced changes of acetylcholinesterase, ATPases and oxidative stress in brain of albino wistar rats. J Ecobiotech 3: 34–9.

Suvarna, K.S.; Layton, C.H. and Bancroft, J.D. (2012). Bancroft's Theory and Practice of Histological Techniques: Expert Consult: Online and Print, 7th Edition. Hardcover. ISBN: 9780702042263

T.H. Reem; Hussain, M.; Ebraheem, K.H. and Hanady, M. (2012). Assessment of heavy metals (Cd, Pb and Zn) con-tents in livers of chicken available in the local markets of Basrah city, IRAQ. Basrah J. Vet. Res., 11(1): 43-51.

Tracey, K.J. (2002). The inflammatory reflex. Nature 420: 853–859. Valko M, Morris H, Cronin MTD (2005) Metals, Toxicity and Oxidative Stress. Curr Med Chem 12: 1161–1208.

Uyanik, F.; Eren, M.; Atasever, A.; Tunoku, G. and Kolsuz, A.H. (2001). Changes in some biochemical parameters and organs of broiler exposed to cadmium and effects of zing on cadmium induced alteration. Israel J. Vet Med 2001; 56(4): 128-34.

Victor, V.M.; Rocha, M. and De la Fuente, M. (2003). N-acetylcysteine protects mice from lethal endotoxemia by regulating the redox state of immune cells. Free Radic. Res 2003; 37: 919-29.

Watson, D. and Rogers, J.A. (1961). "A study of six representative methods of plasma bilirubin analysis". Journal of Clinical Pathology. **14** (3): 271–8.

WHO (2011). Cadmium. Environmental Health Criteria 134. World Health Organisation, International Programme on Chemical Safety (IPCS), Geneva, Switzerland.

Wu, S.M.; Shih, M.J. and Ho, Y.C. (2007). Toxicological stress response and cadmium distribution in hybrid tilapia (Oreochromis sp.) upon cadmium exposure. Comp. Biochem Physiol C **145:** 218-226, 2007.

Xiao, H.L.; Bi, W.X.; Zhi, H.W. and Yao, G.Z. (2018). Characterization and expression analyses of somatolactin-α and -β genes in rare minnows (Gobiocypris rarus) following waterborne cadmium exposure, Fish Physiology and Biochemistry, 10.1007/s10695-018-0487-z, **44**, 3, (983-995), (2018).

Yazıhan, N.; Koçak, M.K.; Akçıl, E.; Erdem, O.; Sayal, A.; Güven, C. and Akyürek, N. (2011). Involvement of galectin-3 in cadmium-induced cardiac toxicity. Anadolu Kardiyol Derg 11: 479–484.

Yin, S,; Sheu, J. and Lin, T. (2000). Lipid peroxidation in rat adrenal glands after administration cadmium and role of essential metals. J Toxicol Env Heal A **62**: 47-56, 2000.

Youssef, A.I. and Mansour, D.H. (2014). Potential role of migratory Quail in spreading of some zoonotic pathogens in Egypt. Am. J. Anim. Vet. Sci., 9(4): 203-210.

Yuan, J.S.; Reed, A.; Chen, F. and Stewart, C.N. (2006). Statistical analysis of real-time PCR data. BMC Bioinformatics, 7:85.

Zhao, Z.; Hyun, J.S.; Satsu, H.; Kakuta, S.; and Shimizu, M. (2006). Oral exposure to cadmium chloride triggers an acute inflammatory response in the intestines of mice, initiated by the over-expression of tissue macrophage inflammatory protein-2 mRNA. Toxicol Lett 164: 144–154.