The Possible Protective Effect of Alpha-Lipoic Acid against Acute Ricin-Induced Nephrotoxicity in Adult Male Albino Rats

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Abstract   **Background:** Ricin toxin is considered one of the most potent plant-derived toxins. It induces rapid and irreversible toxic effects through many mechanisms with special concern to inhibition of protein synthesis and oxidative stress resulting in cell death. Moreover, ricin could be used as a terrorist weapon which indicates the importance of finding a specific treatment. Alpha lipoic acid (ALA) possesses almost all characters of an ideal antioxidant; thereby it is suggested to be used against ricin toxicity. **Aim of the work:** The current study aimed at investigating the acute toxic effects of ricin on the kidneys of adult male albino rats and to appraise the possible protective effect of ALA in altering ricin-induced nephrotoxicity. **Material & Methods:** the present study was carried out on 60 adult male albino rats; they were divided into four groups. Group I**:** ten rats were intraperitoneally injected by 0.9% saline. Group II: ten rats were intraperitoneally injected by 100 mg/kg ALA. Group III**:** twenty rats were subcutaneously injected once with 25 µg/kg body weight of ricin solution. Group IV: twenty rats were injected by 100 mg/kg ALA, 15 minutes prior to single injection of 25 µg/kg body weight of ricin solution, and then reinjected with the same dose of ALA immediately after ricin injection**.** Survival time, renal function tests, renal malondialdehyde (MDA), renal superoxide dismutase (SOD) and catalase enzyme activities were recorded. Kidney samples were used for electron microscopic examination. **Results:** Ricin induced nephrotoxicity with significant increase of renal function tests, renal MDA and catalase activity with significant decrease of SOD. Also, the kidney samples of ricin-treated animals revealed focal ultrastructural changes in renal corpuscles, proximal and distal convoluted tubules. On the other hand, most of ricin-induced injuries were much improved after administration of ALA together with ricin. **Conclusion:** Ricin produces oxidative nephrotoxic effects that markedly improved by the effective antioxidant properties of alpha lipoic acid.

Keywords   Ricin, alpha-lipoic acid, oxidative stress, nephrotoxicity, antioxidant.

Introduction

Ricin toxin (RT) is considered one of the most potent and lethal naturally-occurring substances that ever known; in humans, the median lethal dose of ricin (LD_{50}) after injection or inhalation is about 22 µg/kg of body weight, meanwhile, the estimated lethal oral dose is 1 mg/kg. Hence, some of orally administered ricin is inactivated in the stomach therefore, it becomes less toxic. Meanwhile, ricin toxicity is very limited through intact skin because of its large molecular size and high charge. Ricin is a water-soluble glycoprotein which was firstly extracted from the seeds of castor bean plant (Ricinus communis) by the researcher Peter Hermann Stillmark. Because of its high toxicity, wide availability and the ease of its manufacture, ricin has been considered as one of the biological warfare agents (EFSA, 2008; Moshiri et al., 2016 and Zhou et al., 2017).

The clinical features of ricin toxicity depend on the route of exposure. Oral ricin poisoning causes gastrointestinal (GIT) hemorrhage and necrosis of liver, spleen and kidney. Meanwhile, intramuscular injection causes moderate systemic signs with severe localized pain and necrosis of regional lymph nodes and muscles. Furthermore, ricin inhalation induces respiratory distress with pulmonary and airway lesions. Ricin is known to have rapid and irreversible toxic effects through different possible mechanisms including protein synthesis inhibition, magnesium and calcium imbalances, apoptosis pathways, cytokine release, acute phase reactions, and oxidative stress. Yet, the specific mechanism of ricin toxicity has not been definitively elucidated and the mechanism of death is described as multi-organ failure (Smallshaw & Vitetta, 2012 and Moshiri et al., 2016).

Some selected compounds such as difluoromethylornithine and dexamethasone have been investigated as treatments for ricin poisoning (Poli et al., 2008). Furthermore, many studies tried to develop RT inhibitors but recently more focus has been on producing anti-RT antibodies and monoclonal antibodies for protection of animals against ricin toxicity (Pincus et al., 2011; Barbier et al., 2012 and Chow & Casadevall, 2012). Moreover, the administration of active vaccines has been considered very important for high risk personnel (Zhang et al., 2015).

Despite ricin is known to constitute a bioterrorism toxin, but up-till now, there is no available FDA-approved treatment or vaccine against ricin intoxication. The main line of treatment of ricin toxicity is still good supportive care with standard decontamination methods (Zhou et al., 2017).

Previous studies in the literature revealed that, ricin induces oxidative stress (Muldoon et al., 1994 and Mirakbari, 2015). Therefore, the use of an effective antioxidant has been suggested as a logic treatment for ricin poisoning. Alpha lipoic acid is a popular cofactor of multienzymatic complexes which catalyzes oxidative decarboxylation of α-ketoacids. It fulfills nearly all of features of an ideal antioxidant; as in many tissues lipoic acid is rapidly converted to dihydrolipoic acid (DHLA), its redox couple, and both of them can scavenge free radicals effectively. Also it can regenerates endogenous antioxidants, like glutathione, vitamins E and vitamin C. Moreover, alpha lipoic acid possesses this antioxidant activity in lipid- and aqueous-cell compartments as well (Shay et al., 2009).

Hence, the aim of this work was to investigate the acute toxic effects after subcutaneous injection of 25 µg/kg body weight of ricin on the kidneys of adult male albino rats and to assess the possible protective effect of alpha-lipoic acid (ALA) in altering acute ricin-induced nephrotoxicity.

Material and methods

Chemicals:

Ricin:

At the peak of season; around mid-August, ricin was prepared from extract of ripe castor beans of the communis variety, which were freshly picked from growing shrubs in villages surrounding Tanta city. The average weight of collected seeds was 0.55 gram. The hard thin shells of 180 seeds weighing 100 grams were removed; then, seeds were crushed in a porcelain mortar to make a thick powder. Gradually, we added saline to make a fine emulsion till a total of 500 ml of saline. Later on, this emulsion was kept in a conical flask covered by cotton wool, put on a shaker for 24 hours at 4ºC and was left to sediment for further 48 hours. White sediment was precipitated in the bottom of the conical flask while thick layer of oil was formed on the top of the extract. A large bore long needle was used to carefully aspirate the relatively clear layer in the middle and to transfer it into centrifuge tubes. Centrifugation for 20 minutes at 10000 r.p.m. was performed and repeated till the resultant fluid became clear. Finally, the extract was filtered through filter paper and was stored in 20 ml capacity sterile conical flasks at 4ºC (Eltomey & Abo Hijleh, 1990).

Whereas, standard pure ricin was not available commercially because of its extreme toxicity, the ricin content of the prepared extract was estimated by the fact that approximately each 100 gram of castor beans gave 180 mg of ricin (Woo et al., 1998). Then, a dose of 25 µg/kg body weight of the extract of Castor oil seeds was subcutaneously injected into the experimental animals according to Kumar et al. (2003).

Alpha lipoic acid (ALA):

ALA was purchased as 10 ml ampoule containing 300 mg ALA from EVA Pharma for Pharmaceutical and Medical Appliances, Egypt. A dose of 100 mg/kg body weight of ALA was administered to the experimental animals by intraperitoneal injection (Dulundu et al., 2007).

Experimental animals:

This study was conducted on sixty adult male albino rats of Sprague dawly species supplied by the animal house of the Faculty of Medicine, Tanta University, Egypt. The ages of the experimental animals ranged from 2 to 4 months, and their weights were 125-150 grams. The animals were housed in wire mesh cages with ad-libitum access to food and water under standard conditions of temperature (22-24 ºC) in a 12 hours light/dark cycles.

Ethical considerations

Experimental procedures were performed according to the guide of care and the use of laboratory animals **(NRC, 1996).** The design of the study was approved by the Ethical Committee of Faculty of Medicine, Tanta University, Egypt.

Experimental design:

This study was conducted in Forensic Medicine and Clinical Toxicology Department, Tanta University, Egypt. After one week of acclimatization, rats were divided randomly into four groups:

1- **Group I (Control group):** ten rats were intraperitoneally (i.p.) injected with 0.9% saline.

2 **Group II (ALA- treated group):** ten rats were intraperitoneally injected with 100 mg/kg alpha lipoic acid (ALA).

3- **Group III (Ricin treated group):** it included twenty rats; ten rats were subcutaneously (s.c.) injected once with 25 µg/kg body weight of ricin solution and served to estimate the survival time after ricin injection. The other ten rats were subcutaneously injected once with ricin solution in the same dose and were sacrificed 6 hours before the expected time of death (Kumar et al., 2003)

4- **Group IV (ALA and Ricin treated group):** it included twenty rats; ten rats were injected by 100 mg/kg ALA (i.p.), 15 minutes prior to single injection of 25 µg/kg body weight ricin solution, then reinjected with the same dose of ALA immediately after ricin injection and served to test the survival time after ricin injection. The remaining animals were injected by 100 mg/kg ALA (i.p.), 15 minute prior to single injection of 25 µg/kg body weight ricin solution, then reinjected by ALA in the same dose immediately after ricin injection and will be sacrificed 6 hours before the expected time of death (Dulundu et al., 2007).

Sample collection:

At the appropriate time of each group, animals were sacrificed by cervical dislocation under inhalation of light anesthesia of ether then blood samples were obtained from the heart by needle puncture and kidneys were taken from each animal.

• Blood samples were served to do renal function tests including; serum creatinine (Butler, 1975 and Vasiliades, 1976) and blood urea nitrogen (Fawcett & Scott, 1960 and Chaney & Marbach, 1962).

• Tissue samples from the kidney were obtained and divided into two parts:

- The first part was immediately washed twice in ice cold saline, homogenized in phosphate buffered saline (PBS) with 0.5% triton, and the homogenate was clarified by centrifugation at 800 gm for 15 minutes at 4^0C, then the supernatant was stored at -70 °C till used for determination of malondialdehyde (MDA) concentrations, superoxide dismutase (SOD) and catalase enzyme activities (Yoshioka et al., 1992).

- Renal malondialdehyde (MDA) estimation, the end product of lipid peroxidation, were determined spectrophotometrically at 532 nm using the thiobarbituric acid reactive substances (TBARS) method according to Draper & Hadley (1990).

- Superoxide dismutase (SOD) in renal tissue extract was determined spectrophotometriclly at 560 nm according to Paoletti et al. (1986); which is based on the oxidation of NADH, mediated by superoxide radical. The addition of SOD to the reaction mixture causes proportionate inhibition of the rate of NADH oxidation.

- Determination of catalase activity in renal tissue extract was done spectrophotometriclly at 240 nm according to method for Luck (1956); as per catalase catalyzes the decomposition of substrate (H_2O_2). The amount of peroxide substrate decomposed is directly proportional to concentration of substrate and concentration of the enzyme.

- The second part of tissue samples from the kidney were cut into small pieces 1 mm³ in size and immediately fixed in 2.5% of 0.1 M phosphate buffered glutaraldehyde solution (pH 7.4) at 4° C for 2 hours. After washing of the specimens with phosphate buffer for three times (5 min each time), they were post fixed in 1% phosphate buffered osmium tetra oxide at room temperature for 30 min. Dehydration in a graded series of alcohol (50, 70, 80, 95 and 100%) was carried out followed by washing and embedding in Epon.

- Preparation of ultrathin sections (80–90 nm): The blocks were cut with ultramicrotome, stained with 2% uranyl acetate and lead citrate **(Bozzola & Russel, 1999)** and examined by a JEOL EM at EM Unit, Faculty of Medicine, Mansoura University.

Statistical analysis

The collected data was organized, tabulated and statistically analyzed using SPSS statistical software computer package version 22 (SPSS Inc., Chicago, Illinois, USA). For quantitative data, the Shapiro-Wilk test for normality was performed to assess data distribution. All data were normally distributed and values were expressed as mean ± standard deviation. According to the test of homogeneity of variances, one way ANOVA (followed by Tukey's test) or Welch's ANOVA (followed by Games-Howell test) was performed for comparison between the studied groups. Significance was adopted at $p < 0.05$ for interpretation of results of tests (Dawson-Saunders & Trapp, 2001).

Results
1.   Survival time results
The survival time is determined by the time that passed since injection of rats with 25 µg /kg of ricin till their death. In the present study, the mean survival time in ALA and ricin treated animals (50.8 ± 2.86; range: 47 – 56 hours) was significantly prolonged than the mean survival time recorded in ricin treated animals (23.4 ± 2.41; range: 20 – 27 hours) (p < 0.05).
2.   Biochemical results
2.1.   Renal function tests
Regarding renal biomarkers; serum creatinine and blood urea levels, there were no significant differences in their mean values between group II and group I (control group). The mean blood urea values in groups III and IV showed significant increase when compared to those of control group and ALA treated group (P< 0.001) moreover, this elevation was significantly ameliorated in group IV compared to group III. Concerning serum creatinine; significant increase was detected in the mean serum creatinine of ricin treated animals when compared to groups I and II. However, there was decrease in the mean serum creatinine of group IV compared to that of group III but no significant difference was detected between the two groups III and IV as shown in table (1).
2.2. Renal oxidative stress and antioxidant parameters of the studied groups
Similarly, table (2) revealed that no statistical significant differences were detected in the mean values of renal MDA, SOD and catalase activities between group II and group I (control group). The mean renal MDA showed significant increase in group III as compared to the control and ALA-treated animals. Meanwhile, the administration of ALA with ricin to animals in group IV markedly lowered the increase of renal MDA in group III, but no significant difference was detected between both groups III and IV regarding renal MDA.
On the other hand, the mean renal SOD was significantly decreased in group III as compared to all other studied groups; I, II and IV. Meanwhile, the mean renal catalase showed statistical significant increase in both groups III and IV as compared to groups I and II. Furthermore, there was significant difference between group III and group IV as regards the mean renal catalase values.
3.   Electron Microscope Results:
Groups I (Control group) and II (ALA treated group):
Examination of ultrathin sections obtained from the renal cortex of animals showed the same ultrastructure in both groups I and II. The glomerular blood capillaries appeared formed of fenestrated endothelium with homogenous basement membrane

being formed of outer and inner electron lucent layers and middle electron dense layer. The podocytes had a cell body from which arises cytoplasmic extensions that form primary (major) processes. From these primary processes, secondary (minor) processes or pedicles arise and terminated by feet-like expansions on the basal lamina of the capillary wall. These were minute gaps in-between the minor processes called filtration slits and closed by diaphragms. The cytoplasm of podocytes contained nucleus and organelles like free ribosomes, rough endoplasmic reticulum (RER) and mitochondria (Fig. 1- a & b).

Cells of proximal convoluted tubules (PCT) appeared resting on a thin basement membrane. They showed enfolding of the basal plasma membrane. Numerous thin long apical microvilli were observed forming the characteristic brush border. Each cell of the proximal tubule contained a single large spherical nucleus, nearly central in position, and their chromatin was clumped along the inner nuclear membrane. Mitochondria were numerous elongated with their characteristic palisade arrangement (Fig. 1- c & d).

The cells of distal convoluted tubules (DCT) appeared cubical and had short scattered microvilli on their luminal surfaces. Each cell contained rounded or ovoid nucleus with less extended chromatin and large nucleolus. Large numbers of mitochondria were observed in the basal part (Fig. 1- e & f).

Group III (Ricin treated group):
Examination of ultrathin sections obtained from the renal cortex of animals of group III revealed focal ultrastructural changes in renal corpuscles, proximal and distal convoluted tubules.

Podocytes showed fusion and effacement of their secondary processes, irregular shaped nucleus, dilatation of RER and cytoplasmic vacuolations. The glomerular basement membrane (GBM) was thickened with areas of loss of its trilaminar appearance (Fig.2 a, b and c). The cells of the proximal convoluted tubules showed abnormal shape mitochondria with loss of the normal palisade arrangement, cytoplasmic vacuoles and electron dense bodies (Fig.2d). Some cells of the distal convoluted tubules showed scattered cytoplasmic vacuoles of variable sizes with loss of normal basal arrangement of their mitochondria. Others showed rarified cytoplasm with preservation of the palisade arrangement of mitochondria and even degenerated mitochondria. Discontinuity of the cell membrane of some cells with loss of the most cytoplasmic content could be also observed (Fig.2 e, f, g and h).
Group IV (ALA and ricin treated group):
Examination of ultrathin sections obtained from the renal cortex of animals of group IV revealed

some improvement in the ultrastructural changes in renal corpuscles, proximal and distal convoluted tubules.

The GBM appeared thick in some focal areas with disturbance of its tri-lamellar appearance. Podocytes with fusion of their feet processes, vacuolated cytoplasm and normal shaped nucleus were also found (Fig.3 a, b). The mitochondria of proximal convoluted tubules' cells appeared more or less as the control. Also, dense bodies and cytoplasmic vacuoles were detected inside them (Fig.3c). The cells of the distal convoluted tubules showed more or less normal arrangement of the mitochondria with areas of cytoplasmic rarefaction (Fig.3d).

Table (1): Renal function tests of the studied groups

Studied groups		Serum creatinine (mg/dl)	Blood urea (mg/dl)
		Mean ± SD	Mean ± SD
Group I (Control group)		0.64 ± 0.24	28.3 ± 12.2
Group II (ALA treated group)		0.65 ± 0.26	29.2 ± 13.3
Group III (Ricin treated group)		1.03 ± 0.29	94.7 ± 14.4
Group IV (ALA & ricin treated group)		0.86 ± 0.28	58.9 ± 7.7
One way ANOVA	F	4.797	66.384
	p	0.007*	< 0.001*
	Tukey test	I vs II = 1.000 I vs III = 0.013* I vs IV = 0.278 II vs III = 0.016* II vs IV = 0.317 III vs IV = 0.501	I vs II = 0.998 I vs III < 0.001* I vs IV < 0.001* II vs III < 0.001* II vs IV < 0.001* III vs IV < 0.001*

*ALA: alpha lipoic acid; SD: standard deviation; * significant at $p < 0.05$.*

Table (2): Assessment of renal oxidative/antioxidant parameters of the studied groups

Studied groups		Renal MDA (µM/mg)	Renal SOD (U/mg protein/min)	Renal catalase (U/mg protein)
		Mean ± SD	Mean ± SD	Mean ± SD
Group I (Control group)		55.1 ± 2.9	4.8 ± 1.6	14.4 ± 3.1
Group II (ALA treated group)		54. 2 ± 2.7	4.5 ± 1.5	14.7 ± 2.8
Group III (Ricin treated group)		113.9 ± 17.3	2.5 ± 1.0	18.4 ± 2.5
Group IV (ALA & ricin treated group)		62.6 ± 6.8	4.4 ± 1.3	16.2 ± 2.7
One way ANOVA	F	4.427[a]	5.974[a]	18.557[b]
	P	0.009*	0.002*	<0.001*
	Post hoc test	I vs II = 0.997 I vs III = 0.013* I vs IV = 0.486 II vs III = 0.022* II vs IV = 0.615 III vs IV = 0.287	I vs II = 0.951 I vs III = 0.003* I vs IV = 0.911 II vs III = 0.012* II vs IV = 0.999 III vs IV = 0.017*	I vs II = 0.879 I vs III < 0.001* I vs IV = 0.032* II vs III < 0.001* II vs IV = 0.016* III vs IV < 0.001*

*ALA: alpha lipoic acid; SOD: superoxide dismutase; MDA: malondialdehyde; SD: standard deviation; a: one way ANOVA (post hoc test Tukey test); b: Welch's ANOVA (post hoc test Games-Howell test); * significant at $p <0.05$.*
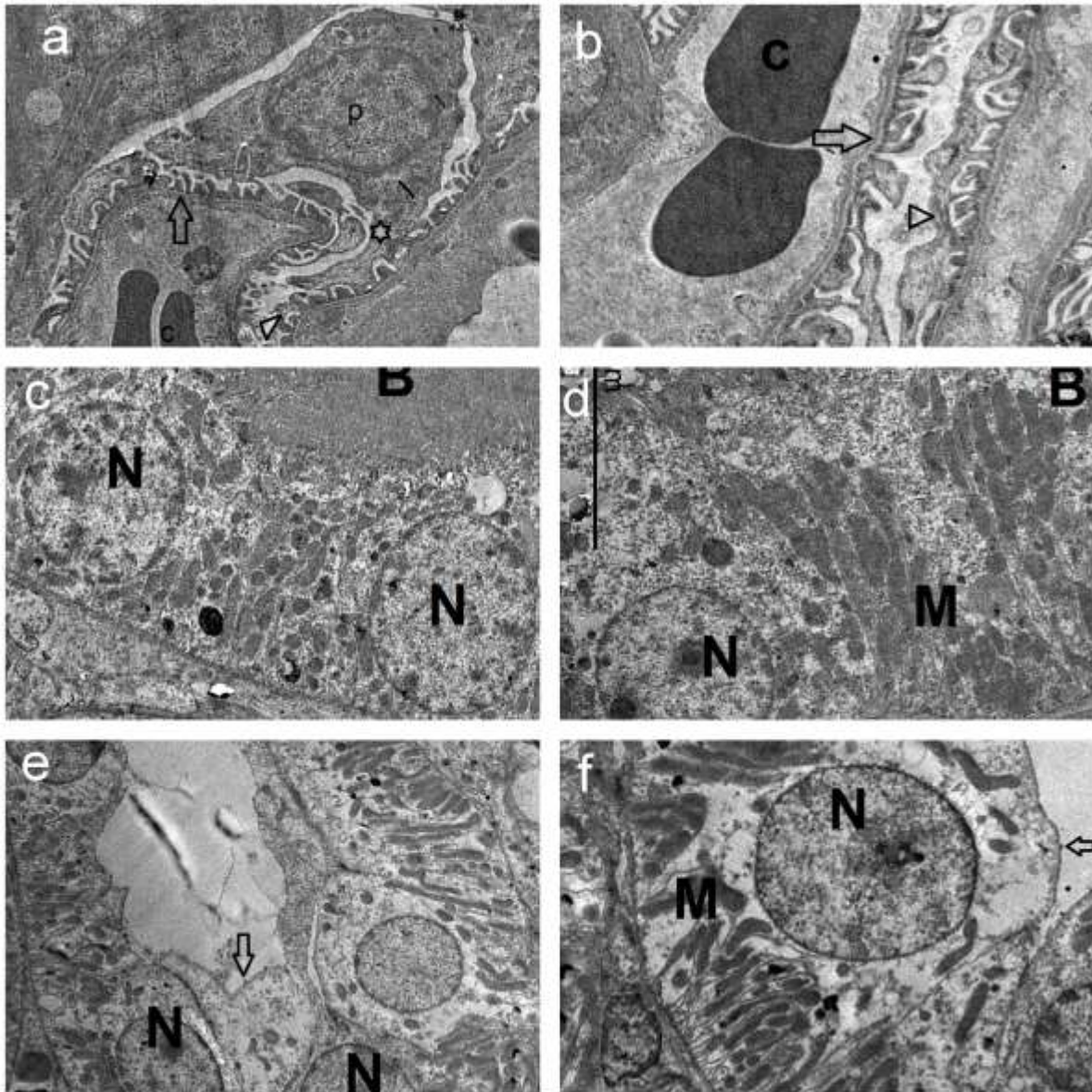
Fig.1: An electron micrograph of a renal cortex from the control group showing; a & b: glomerular blood capillaries (c), GBM with its tri-lamellar appearance and normal thickness (arrows). Normal podocyte cell (p) with its nucleus is found. Notice normal primary (star) and secondary processes (arrow head). c & d: normal PCT cells with its brush border (B), normal nucleus (N) and normal palisade arrangement of mitochondria(M). e & f: normal cells of DCT with normal shaped nucleus (N) and normal arrangement of mitochondria (M). Presence of few apical microvilli (arrows).
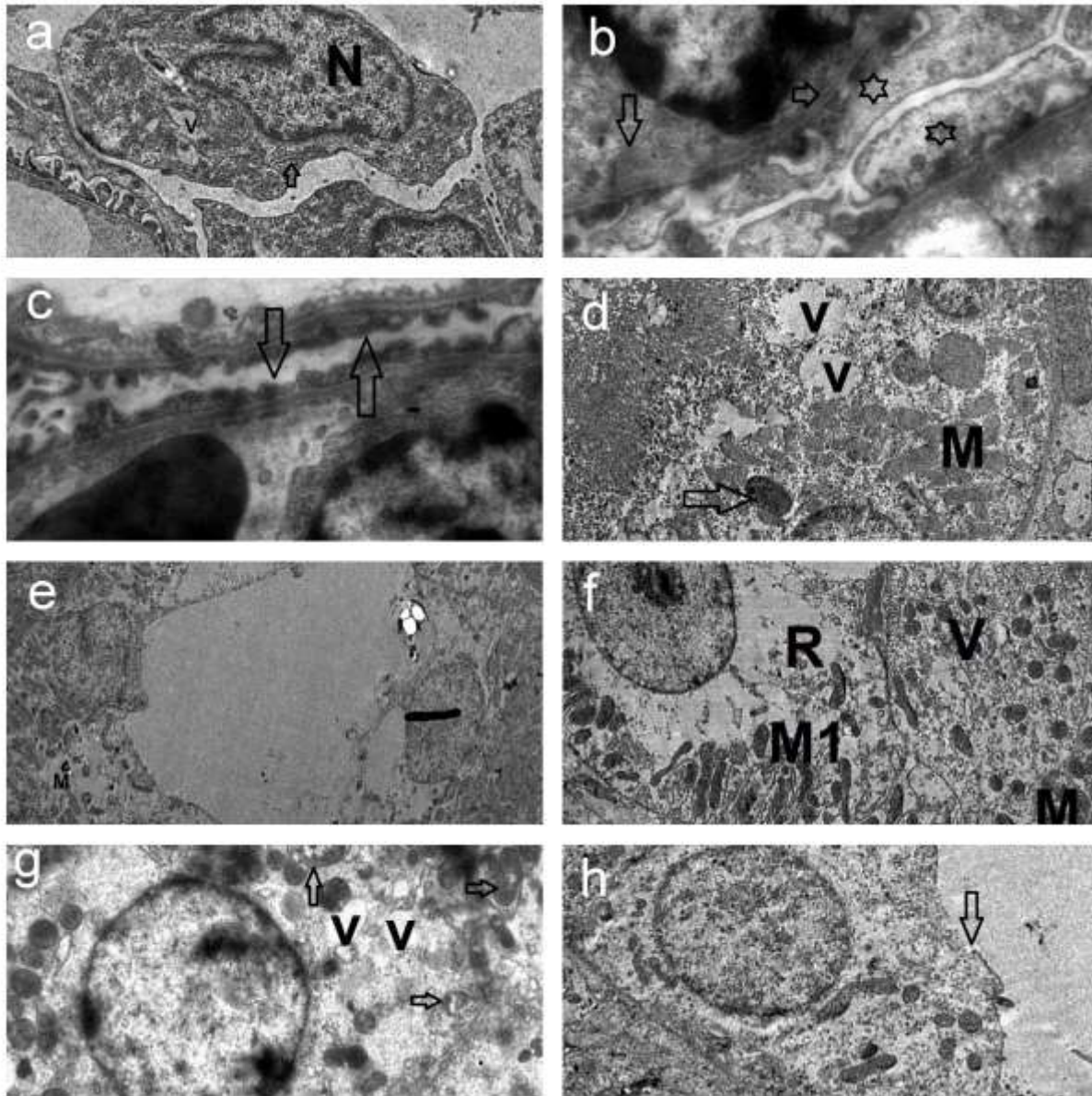
Fig.2: An electron micrograph of a renal cortex from group III showing; a: Podocytes detached from their feet processes with irregular shaped nucleus (N), dilatation of RER (arrows), vacuolation of cytoplasm (v). b: Irregular thickness of the GBM with partial loss its trilaminar appearance (arrows). c: fusion and effacement of feet processes of podocytes (arrows). d: PCT cells with disturbance of the basal arrangement of the mitochondria (M), dense bodies (arrows) and cytoplasmic vacuoles (v). e: DCT cells with rarified cytoplasm (R) and loss of the normal basal palisade arrangement of mitochondria (M). f: DCT cells contained scattered variable sized vacuoles (v), rarified cytoplasm (R) and loss of the normal basal palisade arrangement of mitochondria (M). Some cells showed partial loss of the palisade arrangement of mitochondria (M1). g: DCT cells showed vacuolated cytoplasm (v) and degenerated mitochondria (arrows). h: DCT cell showed discontinuity of its cell membrane (arrow) and loss of most of the cytoplasmic content.
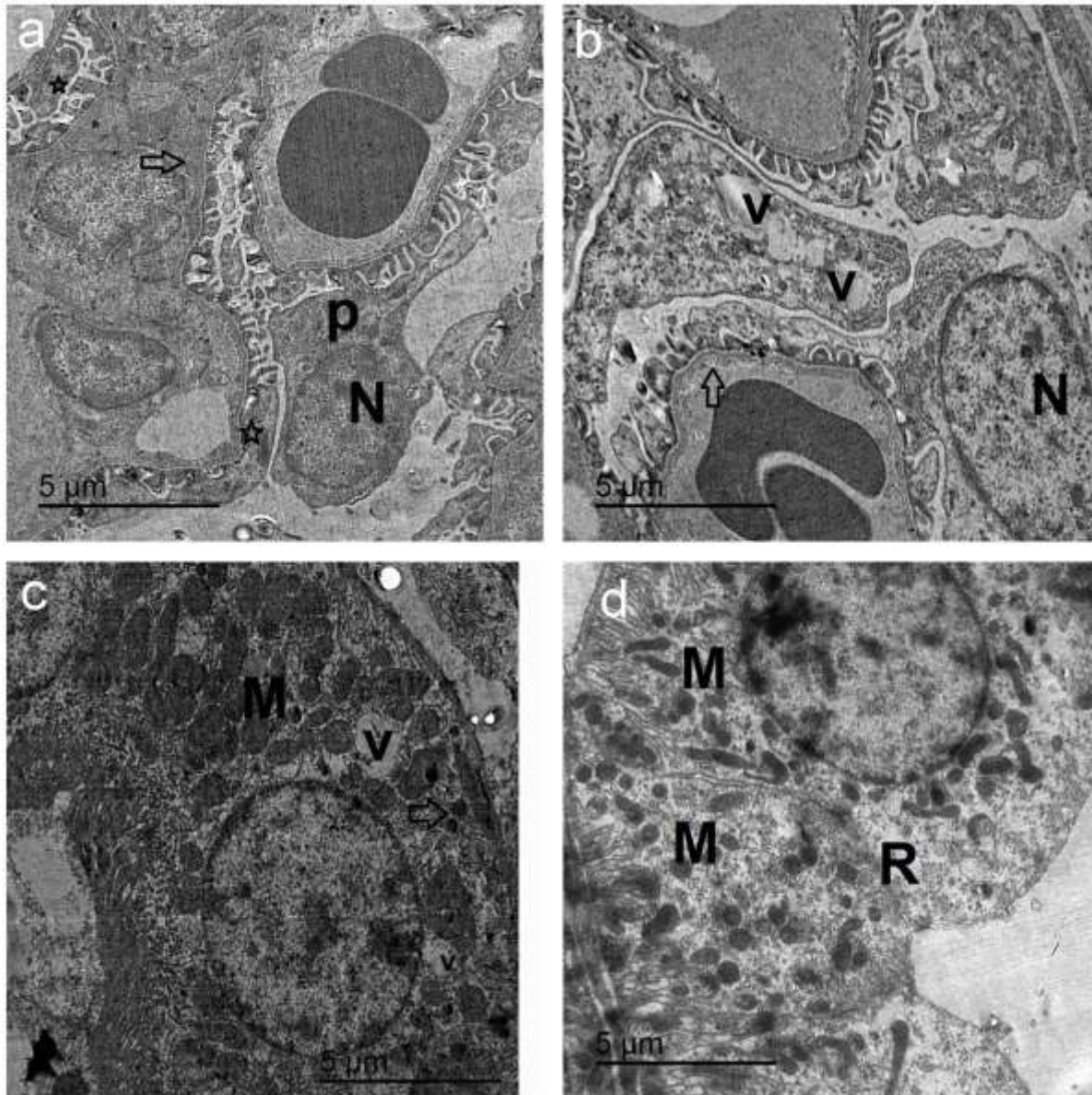
Fig.3: An electron micrograph of a renal cortex from group IV showing; a&b: Thick GBM with loss of its trilamellar appearance (arrow). c: cell of PCT with more or less normal palisade arrangement of the mitochondria (M), cytoplasmic vacuoles (v) and electron dense bodies (arrow). d: cell of DCT showed apparently normal basal palisade arrangement of mitochondria (M) with areas of cytoplasmic rarefaction (R).

Discussion

Ricin has been known as one of the highly toxic plant-derived toxins. The severity of ricin toxicity depends on its route of administration with most potent toxicity through inhalation and least toxicity through oral administration. Generally, the action of ricin toxin is rapid and irreversible which makes its treatment difficult. Moreover, the possible use of ricin as a terrorist weapon indicates the importance of finding specific treatment. Unfortunately, till now there are no FDA-approved specific treatment or vaccines for ricin toxin to be used in human (Lopez-Nunez et al., 2017 and Zhou et al., 2017).

The results of the current study demonstrated that animals subcutaneously injected with ricin died within 20-27 hours which could be elucidated by the mechanisms of action of ricin toxin

causing irreversible inhibition of protein synthesis together with the oxidative stress damage (Yousef et al., 2015 and Noy-Porat et al., 2016).

Hence, ricin is a type 2 ribosome-inactivating protein; it consists of two chains; ricin toxin A (RTA) and ricin toxin B (RTB) chains, both chains are connected by a disulfide bond. RTB binds to the cell surface facilitating the entrance of ricin toxin through endocytosis. In the endoplasmic reticulum, the disulfide bond between the two RT chains is reduced, releasing RTA which enters the cytosol and reaches its target, the ribosome, and has RNA N-glycosidase activity (Balali-Mood & Moshiri, 2015 and Taubenschmid et al., 2017). Ricin inactivates ribosomes by removing a specific adenine residue from the 28S RNA of the 60S ribosomal subunit; thus, protein synthesis is inhibited. One molecule of RTA can inactivate 1500-2000 ribosomes per minute, which resulted in cell morbidity then cell death. The duration between ricin toxicity and the appearance of symptoms was attributed to the time needed to transport ricin into the cell (Sandvig et al., 2010 and Legler et al., 2017).

Oxidative stress has been closely linked to ricin-induced toxicity. This oxidative stress resulted in lipid peroxidation, glutathione depletion and other oxidant mechanisms that damage biological macromolecules specially the cell membrane leading to cell injury and death (Mirakbari, 2015 and Yousef et al., 2015).

On the other hand, the survival time in ALA and ricin treated animals was significantly prolonged which could be attributed to the mechanism of action of alpha-lipoic acid (ALA) which is synthesized in mitochondria, and has an essential role through energy metabolism of mitochondria. ALA possesses not only potent antioxidant activities but also it has free radicals scavenging activities in both reduced and oxidized forms. Moreover, ALA increases tissue levels of glutathione and facilitates the production of vitamin C and E (Shay et al., 2009 and Kim et al., 2016).

In the present study, injecting rats by ricin caused significant increases in blood urea levels and serum creatinine. These results were close to the findings reported by Kumar and his colleagues (2003) who investigated the hepatotoxicity and nephrotoxicity at 24 hours after intra-peritoneal injection of ricin in mice and reported significant elevations of blood urea levels despite creatinine levels were not significantly altered. Moreover, the increased renal function tests results are considered indicators for kidney damage that could be matched with electron microscopic results for renal cortex in the present study including focal ultrastructure changes in renal corpuscles, proximal and distal convoluted tubules.

These results were in agreement with the results of other studies that reported elevated renal biochemical analysis; blood urea nitrogen and serum creatinine levels indicating kidney damage and necrosis after ricin intoxication. It was reported that liver and kidney were most damaged in the initial period of intoxication. These results could be partly elucidated by their abundant blood supply and responsibilities for metabolism and excretion of ricin (Worbs et al., 2011 and Dong et al., 2014). These changes of renal biomarkers could also be explained by damages in the structural integrity of nephrons as it is confirmed that serum creatinine level increases only when at least half of the kidney nephrons are damaged (Khan et al. 2010 and Bellassoued et al., 2018).

Additionally, nephrotoxicity after ricin poisoning has been reported in many human cases in the form of nephritis, hyaline casts on microscopic examination of urine, oliguria, anuria, haematuria and renal failure (Aplin & Eliseo, 1997; Despott & Cachia, 2004 and Assiri, 2012).

Conversely, serum creatinine and blood urea levels were markedly improved on co-administration of ALA with ricin. Obviously, this could be attributed to the potent antioxidant effects of ALA which were also reported by Yang et al. (2017) who showed marked anti-oxidative effects of alpha-lipoic acid in improving methylmercury-mediated neurotoxicity. Similarly, Jeong et al. (2016) described the efficacy of alpha-lipoic acid as an anti-inflammatory, antioxidant, and in reducing cell death in a mouse model after radiation-induced enteritis.

Oxidative stress is defined as imbalance between free radicals production and antioxidant capacity. Living organisms protect themselves against oxidative damage through antioxidant defense system which includes enzymatic and nonenzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione reductase, while nonenzymatic antioxidants include vitamin C (ascorbic acid), vitamin E, urate, betacarotene, carotenoids, polyphenols and selenium among others (Yang et al., 2017). It is obvious that antioxidants perform their action against oxidative stress through decreasing lipid peroxidation and increasing endogenous antioxidant enzymes levels (Bellassoued et al., 2018).

This study showed significant increase in renal malondialdehyde (MDA) and catalase activity

with concomitant decrease in renal SOD activity after injection of rats with ricin, which elucidates that ricin produces oxidative stress in the kidney. These results coincided with the results of previous studies in the literature which reported that ricin toxicity induces oxidative stress leading to cell injury through energy metabolism disturbance, mitochondrial membrane disruption, lipid peroxidation and glutathione depletion (Muldoon et al., 1994; Kumar et al., 2007 and Guo et al., 2014)

The increased MDA means increased lipid peroxidation as MDA is the end product of lipid peroxidation and one of the compounds produced by decomposition of peroxidized polysaturated fatty acids (Zhou et al., 2017). Moreover, SOD catalyzes the reaction of two (O_2^-) molecules with (H^+) ions to form hydrogen peroxide (H_2O_2) and superoxide radicals $(O_2^{- \cdot})$. Hence, ricin toxicity resulted in overproduction of superoxide radicals and H_2O_2 causing some sort of SOD enzyme exhaustion in ricin treated animals. These results were close to the results reported by Bellassoued et al. (2018) who studied carbon tetrachloride (CCl_4)-induced oxidative damage in liver and kidney of rats.

Unexpectedly, in ricin treated group, the catalase enzyme activities were significantly elevated than in the ALA and ricin treated group. This could be explained by direct relationship between increased free radicals associated with oxidative stress and the increased antioxidants enzyme activities as per catalase is used to catalyze the detoxification of hydrogen peroxide to water and oxygen as a defense mechanism against ricin-induced oxidative stress (Delcarmen et al., 2002). These results were in agreement with the results of Kumar et al. (2003 and 2007).

Interestingly, the current study showed marked decrease in renal oxidative stress as evidenced by reduced MDA levels in kidney tissues and improvement in antioxidants activities including elevation of SOD and decrease of catalase enzymes activities in animals treated with ALA and ricin. These results could be attributed to the effective antioxidant activities of ALA and also its free radicals scavenging properties. Similar results were reported by other studies (Shay et al., 2009; Jeong et al., 2016 and Yang et al., 2017).

The damaging effect of ricin on kidney was evidenced by the renal biochemical changes that support the electron microscopic findings of the renal cortex observed in this study, where animals in ricin treated group showed focal ultrastructural changes in renal corpuscles, proximal and distal convoluted tubules. These changes included fusion of podocytes

and effacement of their secondary processes, irregular shaped nucleus, dilatation of RER, cytoplasmic vacuolations, thickened GBM. Also, proximal convoluted tubular cells showed abnormal shape mitochondria, cytoplasmic vacuoles and electron dense bodies. Some distal convoluted tubular cells showed scattered cytoplasmic vacuoles with loss of normal basal arrangement of their mitochondria. Others showed rarified cytoplasm with degenerated mitochondria.

Reference wise, the mechanism beyond ricin-induced ultrastructural changes in the kidney cells could be attributed to inhibition of protein synthesis after ricin poisoning leading to alteration in normal turn-over process of glomerular membrane proteins resulting in thickening of the glomerular basement membrane (Chishti & Rotkiewicz, 1992). Also, renal haemodynamic disturbances resulted from ricin- induced vascular congestion could contribute to thickening of the glomerular basement membrane (Ekladious et al., 1992).

Furthermore, the dilatation of RER in ricin treated rats could be a sign of protein synthesis inhibition as Vikas and Bhatia (1990) suggested that RER dilatation could be an adaptive process to increase protein synthesis in response to RNA reduction associated with ricin-induced cellular damage.

Moreover, Elsayed (1994) reported that the increased blood urea levels and serum creatinine observed in ricin treated group could be elucidated by the alteration of the podocytic pores resulting in disturbances of the glomerular filtration leading to abnormal renal function tests.

Additionally, this study revealed the appearance of secondary lysosomes which was in agreement with Chishti & Rotkiewicz (1992) who found that any toxic exposure is always associated with appearance of secondary lysosomes as an attempt to sequestrate or auto-phage the damaged organelles by the toxin.

The improvement of renal function tests and oxidative/antioxidant parameters correlated with more or less normal renal electron microscopic findings observed in rats of group IV. These results indicating that the administration of ALA with ricin protected the kidney of rats from the oxidative injurious effect of ricin. Similar results were found by other studies that revealed the protective antioxidant effects of ALA in prevention of toxin- induced oxidative injuries (Jeong et al., 2016 and Yang et al., 2017)

In conclusion, the current study reveals that ricin induces oxidative stress resulting in kidney

injury. Alpha lipoic acid possesses the ability to act as an effective antioxidant that markedly ameliorates ricin-induced nephrotoxicity. However, further clinical studies are recommended to clarify its safety and to declare its clinical application.

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<u>**الملخص العربي**</u>

التأثير الوقائي المحتمل لحمض الألفا ليبويك ضد التسمم الكلوي الحاد بالريسين في ذكور الجرذان البيضاء البالغة

دعاء محمد الغرباوي ومروة محمد شاهين[1] و صديقة محمد توفيق[2] و أحمد نبيل محمد زكي[3]

المقدمة: يعتبر سم الريسين واحدا من أكثر السموم المستمدة من النباتات فعالية حيث يسبب تأثير سام دائم و سريع وذلك من خلال العديد من الآليات خاصة تثبيط تخليق البروتين والإجهاد التأكسدي مما يؤدى إلى موت الخلايا. بالإضافة الى إمكانية استخدام الريسين كسلاح إرهابي مما يبرز أهمية إيجاد علاج له. و يمتلك حمض الألفا ليبويك معظم الصفات المثالية كمضاد للأكسدة ، ولذلك فمن الممكن استخدامه ضد تسمم الريسين.

الهدف من البحث: أجريت الدراسة الحالية لمعرفة التأثير السام للريسين على الكلى فى ذكور الجرذان البيضاء البالغة وتقييم التأثير الوقائي المحتمل لحمض الألفا ليبويك فى تعديل التسمم الكلوي الناتج عن الريسين.

طريقة البحث: أجريت الدراسة الحالية على ٦٠ من ذكور الجرذان البيضاء البالغة ؛ و قد تم تقسيم الجرذان إلى أربعة مجموعات. المجموعة الأولى: عشرة جرذان تستخدم كمجموعة ضابطة وتم اعطائهم ٠,٩٪ محلول ملحى عن طريق الحقن داخل البريتون. المجموعة الثانية: عشرة جرذان وتم اعطائهم ١٠٠ ملليجرام / كجم من حمض الألفا ليبويك عن طريق الحقن داخل البريتون. المجموعة الثالثة: تم حقن عشرين من الجرذان مرة واحدة ب٢٥ ميكروجرام / كجم من الريسين تحت الجلد. أما المجموعة الرابعة: فقد تم حقن عشرين من الجرذان ب١٠٠ ملليجرام / كجم من حمض الألفا ليبويك ١٥ دقيقة قبل حقن الريسين وأيضا بعده مباشرة. وقد تم تسجيل مدة بقاء الجرذان على قيد الحياة، واختبارات وظائف الكلى، و مستوى المالونديالدهيد الكلوي ، ونشاط كل من انزيم السوبر أكسيد ديسميوتيز و انزيم الكاتالاز بالكلى. كما تم أخذ عينات الكلى لإجراء الفحص المجهري الإلكتروني.

النتائج: أدى حقن الريسين الى تسمم كلوي صاحبته زيادات ذات دلالات احصائية في اختبارات وظائف الكلى، و مستوى المالونديالدهيد الكلوي ونشاط انزيم الكاتالاز مع انخفاض ذو دلالة احصائية فى انزيم السوبر أكسيد ديسميوتيز. كما كشف الفحص المجهرى الالكترونى لعينات الكلى المأخوذة من الحيوانات المعالجة بالريسين عن تغيرات البنية التركيبية في الكريات الكلوية، والأنابيب الملتوية بالكلى. بينما على الجانب الاخر، فإن معظم الإصابات الناجمة عن الريسين تحسنت تحسنا كبيرا بعد اعطاء حمض الألفا ليبويك مع الريسين.

الاستنتاج: و قد خلصت الدراسة الى ان الريسين يسبب تسمم كلوي يصاحبه إجهاد تأكسدي و لكن هذا التسمم قد تحسن بشكل ملحوظ عند استخدام حمض الألفا الليبويك والذى يمتلك خصائص فعالة كمضاد للأكسدة.

الكلمات المفتاحية: الريسين، حمض الألفا ليبويك، إجهاد تأكسدي ، تسمم كلوي، مضاد الأكسدة.

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