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# Acrylamide induced histochemical and immunohistochemical alterations in rat kidney cortex

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## Abstract

Acrylamide represents an industrial chemical and has become one of the main public health concerns since it was detected in extensively consumed food items. The present study was planned to investigate the effects of two doses of acrylamide on some enzyme activities and immunoreactivity of some immunohistochemical parameters in kidney cortex of male rats. Rats were randomly divided equally into three groups. Group (I) was control, group (II) was given acrylamide for 6 months orally in a dose of 0.05 mg/ /Kg dissolved in water for 3 doses per week and group (III) was administered acrylamide in a dose of 0.5 mg by the same way as in group II. At the end of the experiment all animals were sacrificed under anesthesia, kidney was immediately removed and processed for histochemical and immunohistochemical studies. The results revealed that the activities of lactic dehydrogenase (LDH) and xanthine oxidoreductase (XOR) were significantly increased, while succinic dehydrogenase (SDH) activity was decreased significantly compared to control group. On the other hand, immunohistochemical results showed that acrylamide significantly reduced the immunoreactions of endothelial nitric oxide synthase (e-NOS) in addition to significant increase in immunoreactivity of inducible nitric oxide synthase (i-NOS) and insignificant increase in alpha smooth muscle actin (α-SMA) with respect to control group. Over all, these results suggested that the deleterious effect in kidney tissue resulted from oral administration of acrylamide, most probably due to oxidative stress and lipid peroxidation.

***Keywords*:** Acrylamide, α-SMA, LDH, SDH, NOS, XOD.

## 1 Introduction

Acrylamide or 2-propenamide (ACR) an industrial chemical formed in some foods particularly starchy foods during heating process such as baking, frying and roasting. High acrylamide levels were found in fried or baked starchy foods such as cereals, biscuits, French fries and potato chips (Tareke et al., 2002).

ACR is formed in foods by the reaction of the free amino acid, asparagines, with reducing sugars (glucose and/or fructose) as part of the Maillard reaction during heating under high temperature and low moisture conditions (Krishnakumar and Visvanathan, 2014). ACR administration caused disturbances in the oxidative status and enzyme activities in the liver of rat which were pronounced with the high doses (Teodor et al 2011). Mice were administrated IP with ACR in a dose of 40 mg/kg b.w/ d for 5days induced significant oxidative damage in the brain cortex and liver as evidenced by elevated lipid peroxidation, reactive oxygen species (ROS) and protein carbonyls. The authors attributed these effects to lowered antioxidant activities including antioxidant enzymes (catalase, glutathione-s-transferase) and reduced glutathione (GSH) compared to untreated controls (George et al., 2013).

ACR induced oxidative stress on membrane polyunsaturated fatty acids in rat's stomach, liver and kidney after supplemented with 0.05% ACR in drinking water for forty day (Sadek, 2012).

Oxidative stress has a critical role in the pathophysiology of several kidney diseases, and many complications of these diseases are mediated by oxidative stress, oxidative stress-related mediators, and inflammation. The well known ROS are superoxide ion ($O_2$ •−), hydrogen peroxide ($H_2O_2$) and peroxyl radical (OH•), while the reactive nitrogen species (RNS) are nitric oxide (NO) and peroxynitrite ($ONOO^-$) (Balaban et al 2005**).** Oxidative stress is mainly linked to mitochondrial dysfunction, as mitochondria are either generators or targets for reactive species (Murphy , 2009) and peroxisomal fatty acid, cytochrome P-450, and phagocytic cells (Balaban et al., 2005).

The kidney is an organ highly susceptible to damage caused by ROS, likely due to the abundance of long chain polyunsaturated fatty acids in the composition of renal lipids. Oxidative stresses have become one of the most popular topics in research of molecular mechanism of renal diseases. (Ozbek, 2012).

Succinic dehydrogenase (SDH) is a key enzyme in the Krebs cycle and the activity level of which shows the degree of the activity of mitochondria ( Nakatani et al., 1999). Succinic dehydrogenase (SDH) oxidizes succinate to fumarate as a component of the tricarboxylic acid cycle and ubiquinone to ubiquinol in the mitochondrial electron transport chain ( Huang and Millar, 2013).

Lactate dehydrogenase (LDH) is a terminal glycolytic enzyme that plays an indispensable role in the inter-conversion of pyruvate to lactate to yield energy under anaerobic (Kavanagh et al., 2004) and the reaction occurs in both cytosolic and mitochondrial compartments (Tabouy et al., 1998)

Xanthine oxidoreductase exists in two functionally distinct forms; xanthine oxidase (XO) and xanthine dehydrogenase (XDH). Under normal conditions, the larger part of the enzyme occurs as an NAD $^{(+)}$-dependent dehydrogenase form which produces NADH and urate. The dehydrogenase can be transformed under various pathophysiological conditions to an oxygen-dependent oxidase form which produces oxygen radicals and/or hydrogen peroxide and urate (Frederiks and Marx ,1993).

Smooth muscle actin (SMA) is a microfilamentous contractile protein. Alpha-smooth muscle actin (α-SMA) is the actin isoform that predominates within vascular smooth-muscle cells and plays an important role in fibrogenesis (Cherng et al., 2008) and correlates with the activation of myofibroblasts (Micili et al., 2013). Chronic nephrotoxicity is characterized by irreversible interstitial fibrosis, which usually leads to impaired renal function (Burdmann et al., 2003).

Nitric oxide (NO) radicals are recognized as important mediators in various physiological and pathophysiological processes. NO radicals are synthesized from L-arginine by nitric oxide synthase (NOS) of which three isoforms can be distinguished: (*1*) neuronal or brain NOS (nNOS, type I), (*2*) inducible NOS (iNOS, type II), and (*3*) endothelial NOS (eNOS, type III) (Moncada and Higgs, 1993). Physiologically, NO is important not only in the regulation of renal hemodynamics but also in the regulation of renal tubular function ( Radermacher, et al., 1992 ). High levels of NO produced by iNOS have been implicated in the renal dysfunction/injury associated with either renal ischemia/reperfusion injury (Yokozawa et al. ,1999) or during endotoxic and hemorrhagic shock (Thiemermann et al., 1995 and McDonald et al., 2001). In addition, the cytotoxic $ONOO^-$ lead to increased renal injury which might damage the tubular cells resulting in renal failure (Walker et al., 2000). eNOS- derived NO has been known to have a pivotal role in the modulation of vascular tone and function (Zhang et al., 2009). eNOS was mainly indicated to be associated with cell death (Hellberg et al., 1998).

The current study was designed to perceive the harmful effect of chronic intake of acrylamide on some histochemical and immunohistochemical parameters in kidney cortex of male rats.

## 2 Materials and Methods

This study was carried out in accordance with the protocol of Laboratory Animal Unit of Medical Research Institute, Alexandria University concerning with guiding principles for biomedical research involving animals.

The current study was applied on 30 adult male albino rats weighing 130-150 g obtained from the Laboratory Animal Unit, Medical Research Institute, Alexandria University. They were kept under the same laboratory conditions of 20–25 $^0$C and 12 h light–dark cycle and given free access to standard food and water. Rats were then randomly divided equally into 3 groups. Group I: ten rats were served as normal control. Group II: ten rats were administered acrylamide (Sigma Chemical Company) orally by gastric tube in a dose of 0.05 mg, dissolved in water /Kg body weight for 3 doses, every other day, per week for 6 months (Hassan, 2005). Group III: ten rats were administered acrylamide in a dose of 0.5 mg by the same way as in group II.

At the end of the experiment, all animals were sacrificed under anesthesia. Kidney was immediately removed and divided into 2 parts; one part was fixed in 10% neutral buffered formalin for preparing paraffin sections and the other part was used as frozen cryostat sections. Then the following studies were performed:

**Enzyme Histochemical Studies**

For all enzymes histochemical reactions 10 µm-thick fresh frozen cryostat sections were used. Stained sections from all studied groups were compared with their negative control sections which were obtained by using incubating medium without substrate.

**Succinic dehydrogenase (SDH):** It was studied according to the method of Pearse (1972). Frozen sections were incubated for 1 hour at 37$^o$C in a medium

containing: 1 ml 0.2M sodium succinate, 2.5 ml 0.2 M tris buffer pH 7.4, 1 ml 0.05 M magnesium chloride, 10 mg nitroblue tetrazolium, 3 ml disilled water. Sections were then fixed in 15% formal- saline for 15 minutes, washed in running water, dried and mounted in glycerin jelly.

**Lactic dehydrogenase (LDH):** For demonstration of lactic dehydrogenase,

frozen sections were incubated for one hour at 37$^{o}$C in a medium containing :1 ml 0.1M sodium lactate, 2.5 ml 0.2 M tris buffer pH 7.4, 1 ml 0.05 M magnesium chloride, 10 mg nitroblue tetrazolium, 3 ml disilled water and 10 mg nicotinamide adenine dinucleotide. Sections were then fixed in 15% formal- saline for 15 minutes, washed in running water, dried and mounted in glycerin jelly ( Pearse, 1972).

**Xanthine oxidoreductase (XOR):** Demonstration of xanthine oxidoreductase activity was performed by incubating frozen sections for 20 minutes at 37$^{0}$C with media containing 18% (w/v) polyvinyl alcohol , 0.1 mol/L of phosphate buffer, pH 8.0; 5 mmol/L of nitro blue tetrazolium; 0.45 mmol/L of 1-methoxyphenazine methosulfate; and 0.5 mmol/L of hypoxanthine. Negative Control sections were obtained by incubation in the absence of hypoxanthine. After incubation, sections were washed in 0.1 mol/L of phosphate buffer, pH 5.3, at 60$^{o}$C to stop the reaction immediately and to rinse off the viscous media. Sections were embedded in glycerol jelly (Kooij et al., 1991).

**Immunohistochemical studies:**

Immunohistochemical studies of alpha smooth muscle actin (α-SMA), endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in kidney sections were performed according to the following technique:

Formalin fixed paraffin sections of 4μ thick were brood onto coated glass slides. Sections were deparaffinized in xylene, rehydrated and rinsed in phosphate buffered saline (PBS). Sections were incubated in 3% $H_20_2$ in methanol for 10 minutes to inhibit endogenous peroxidase activity, and blocked with nonspecific staining blocking reagent. Sections were incubated overnight at 4$^{0}$C with anti-α-SMA antibody (mouse monoclonal antibody-diluted 1:100; Sigma) (Skalli et al., 1986), or with anti-eNOS antibody (rabbit polyclonal -2 μg/mL diluted in PBS with 1.5% normal goat serum ; BioGen, RB-9279-R7, USA) (Lamas et al, 1992) or with anti-iNOS antibody (rabbit polyclonal at 1:100 dilution, ab15323) (Goto et al., 1995). For the negative controls, PBS was used in place of the primary antibody. After incubation, sections were processed according to the standard immunoperoxidase method, using a streptavidin biotin peroxidase complex kit (Sigma Co). The immunohistochemical reaction was then developed and stained with diaminobenzidine chromogen solution "DAB" (Sigma). Sections were counter- stained with hematoxylin, dehydrated, cleared, and mounted with DPX.

**Image analysis**

Five digital images of kidney sections from each studied group were analyzed with a semi-quantitative scoring system (Image J software, Java based application for analyzing images). For both histochemical and immunohistochemical reactions, the colored stained area according to each reaction was examined. The mean percentage of stained area per field area was calculated. The percentage of area occupied by each enzyme reaction as well as total immunostained (brown) was determined according to the following equation, area % = (stained area /total image area) × 100.

Data which obtained by image analysis was expressed in (Mean. ± SD) and was compared using F (ANOVA) test. Significant between groups was done using Post Hoc test (LSD) and the significant level was defined at $p \leq 0.05$.

## 3 Results

**Histochemical results**

Succinic dehydrogenase (SDH) activity was observed as fine bluish violet formazan granules distributed in the cytoplasm of epithelial cells in proximal and distal convoluted tubules in kidney cortex referring to mitochondria. Examined kidney sections showed strong homogenously distributed SDH activity in normal kidney tubules as well as moderate and weak activity after low and high doses of acrylamide administration respectively (Figure 1).

Lactic dehydrogenase (LDH) activity was seen as violet staining granules pronounced in the cytoplasm of epithelial cells of proximal and distal convoluted tubules in kidney cortex. Normal sections exhibited evenly distributed weak enzyme activity in kidney tubules. After acrylamide administration, the increment in LDH activity was dose dependent; revealed moderate and strong (Figure 2).

Xanthine oxidoreductase (XOR) activity was observed as fine blue granules in kidney tubules with weak intensity in sections of control animals. After acrylamide administration, kidney sections revealed moderate and strong enzyme activity after low and high doses respectively (Figure 3).

The changes in enzymes' activities in the three studied groups as analyzed by Image J which represented by the mean of area percentage are summarized figure (4). The results recorded a significant decrease in the mean of area percentage of SDH after administration with low (22.26 ± 3.12) and high (19.77 ± 2.54) doses of acrylamide with respect to the control (29.38 ± 3.57) at $p \leq 0.05$. However the activity of LDH recorded a significant increase in

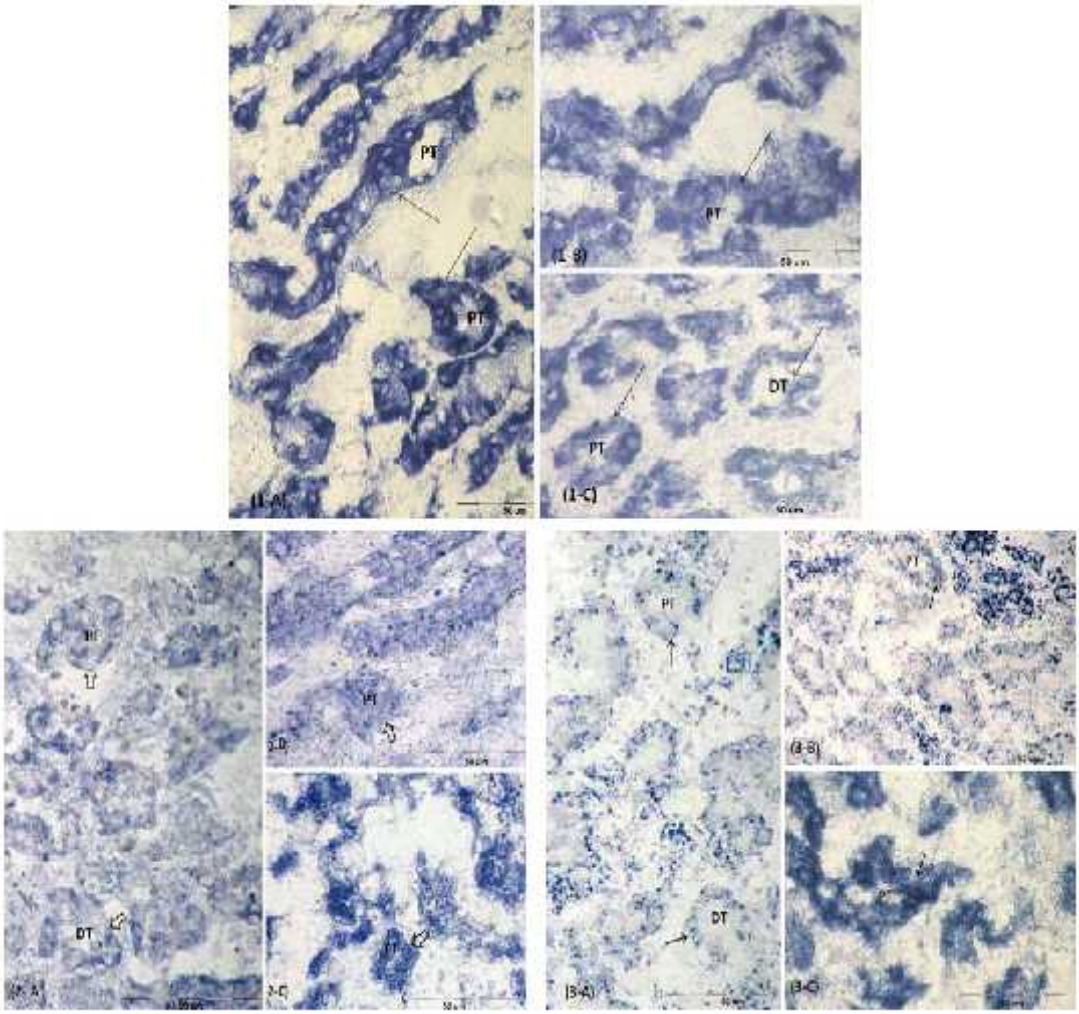


Figure 1: Photomicrographs of the kidney cortex sections showing (SDH) activity in tubular cells. **a)** strong activity (↑) in proximal tubules (PT) in control kidney, **b)** moderate activity in group II and **c)** diminished enzyme activity (↑) in distal (DT) and proximal tubules (PT) in group III . (Bar= 50µm)

Figure 2: Photomicrographs of kidney cortex sections showing LDH activity; **a)** Evenly distributed weak LDH activity (⇧) in the epithelial cells of the proximal (PT) and distal tubules (DT) in control kidney sections, **b)** Irregular coarse granules of enzyme with moderate activity (⇧) in proximal tubules (PT) in kidney sections of group II, and **c)** Strong enzyme activity (⇧) in proximal tubules (PT) in kidney sections of group III. (Bar = 50 µm)

Figure 3: Photomicrographs of the kidney cortex sections showing xanthine oxidoreductase ; a). Weak enzyme activity (↑) in proximal (PT) and distal tubules (DT) in control kidney sections, (b) moderate coarse granules of enzyme activity (↑) in proximal tubules (PT) of kidney sections of group II and (c) strong enzyme activity (↑) in proximal tubules (PT) of kidney sections in group III. (Bar = 50 µm)

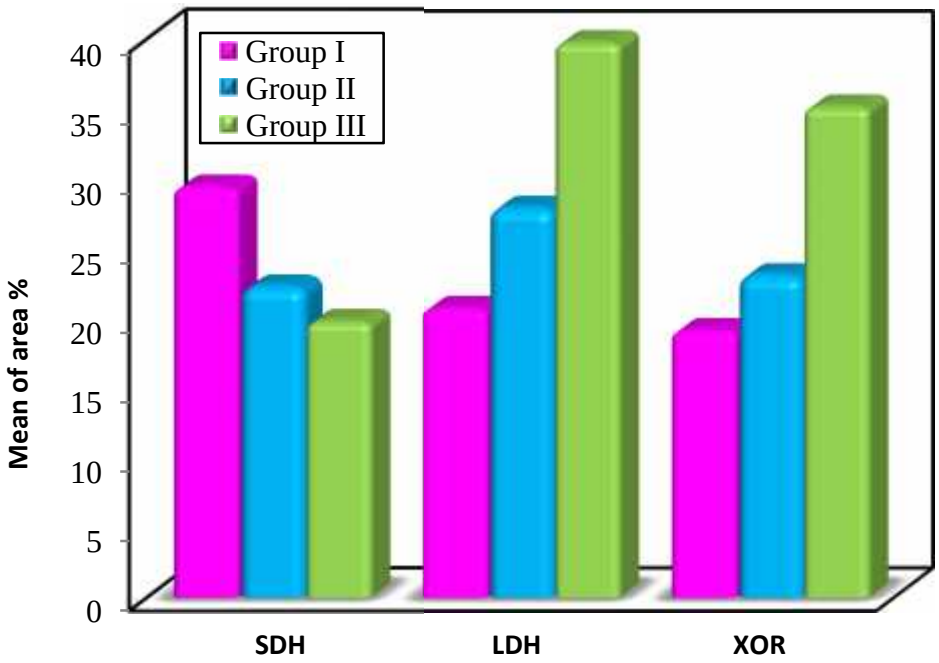


**Figure 4: Bar graph showing different enzymes' activities represented by mean of area percentage in the three studied groups**

mean area percentage after high dose of acrylamide (39.86 ± 7.06) as compared with low dose (27.99 ± 3.77) and control (20.77 ± 5.27) at $p \leq 0.05$.The high dose of acrylamide revealed significant increase in mean of area percentage of XOR activity (35.24 ± 6.51) as compared with each of low dose (23.15 ± 4.26) and control (19.17 ± 3.76) at $p \leq 0.05$.

**Immunohistochemical results**

Immunostaining reaction of α-smooth muscle actin (α-SMA) was visualized in paraffin kidney sections from all groups. It was indicated as brown color in vascular smooth- muscle cells in kidney cortex. Our results showed minimal increase in α-SMA expression in kidney sections after acrylamide administration comparing with normal control as seen in figure (5).

Immunohistochemical expression of endothelial nitric oxide synthase (eNOS) in the kidney cortex was seen as brown staining color in cytoplasm of proximal and distal tubules and no reaction staining in glomeruli. Control sections revealed moderate immunoreactivity in cytoplasm of most proximal and distal tubules. Weak positivity of e-NOS expression was recorded in tubular cytoplasm of kidney sections after acrylamide administration (Figure 6).

Immunohistochemical expression of inducible nitric oxide synthase (i-NOS) in kidney cortex was observed as brown stain in proximal tubular epithelial cells. Moderate immunostaining of i-NOS was found in control sections as well as after low dose of acrylamide and strong i-NOS immunoexpression was recorded after high dose of acrylamide (Figure 7).

The changes in the expressions of immunoreactions of α-SMA, e-NOS and i-NOS in the three studied groups as analyzed by Image J which represented by the mean of area percentage were summarized in figure (8).

The mean area percentage of brown-stained immunoreaction of α-SMA showed no significant difference ($p > 0.05$) after acrylamide administration with low (9.79 ± 1.62) and high dose (10.33 ± 2.12) as compared with control (9.10 ± 1.54).

Moreover, the mean area percentage of e-NOS immunoreactivity declared statistically significant lower immunoreaction ($p \leq 0.05$) in kidney sections of low dose (12.97 ± 2.40) and high dose (10.40 ± 1.22) of acrylamide as compared with control sections (16.54 ± 2.11).

Also the mean area percentage of i-NOS immunoreactivity revealed higher significant difference ($p \leq 0.05$) after high dose of acrylamide (38.03 ± 6.89) as compared with each of treated with low dose (27.41 ± 5.73) and control (25.72 ± 4.24).

## 4 Discussion

It is well known that oxidative stress plays an essential role in the development of acrylamide nephrotoxicity (Morsy et al., 2014). Oxidative stress has a critical role in the pathophysiology of several kidney diseases, and many complications of these diseases are mediated by oxidative stress, oxidative stress-related mediators, and inflammation (Ozbek, 2012).

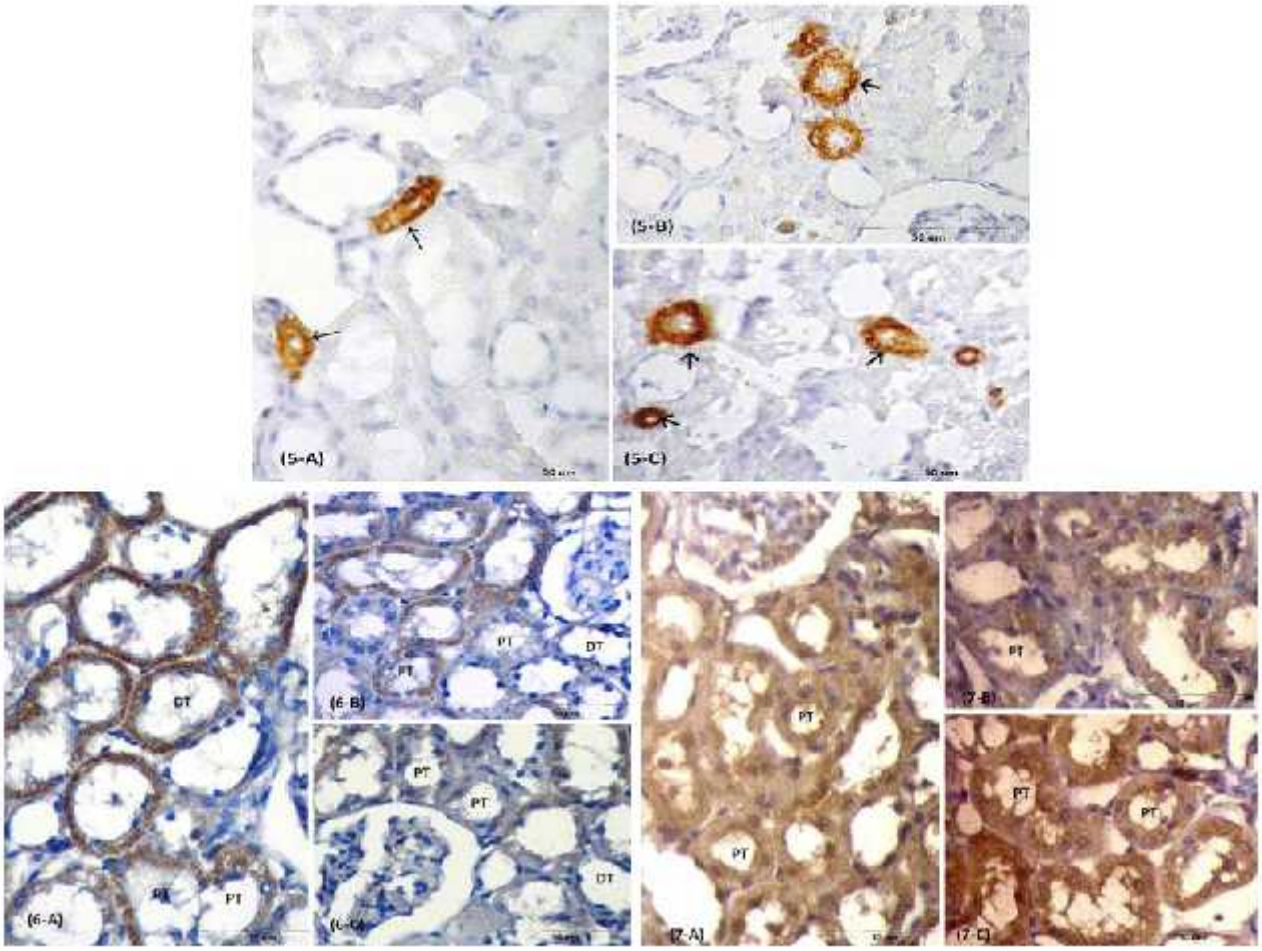


Figure 5: Photomicrographs of immuno-localization of α-smooth muscle actin in sections of kidney cortex. **a)** Normal expression of α-SMA (↑) in vascular smooth muscle cells. **b)** and **c)** Minimal proliferation of α-SMA expression (↑) in vascular cells in kidney sections of group II and III respectively. (Bar = 50 µm).

Figure 6: Photomicrographs of immuno-localization of endothelial nitric oxide synthase (e-NOS) in sections of kidney cortex. **a)** Group I has moderate immunoreactivity in cytoplasm of most proximal and distal tubules. **b) and c)**: Weak positivity of e-NOS expression in tubular cytoplasm of kidney sections in group II and group III respectively. (Bar = 50 µm).

Figure 7: Photomicrographs of immuno-localization of inducible nitric oxide synthase (i-NOS) in sections of kidney cortex. **a) and b)** Moderate immunoreactivity in proximal tubular epithelial cells (PT) in groups I and II. **c)** Strong immune-reaction in proximal tubules of kidney sections of group III. (Bar = 50 µm).

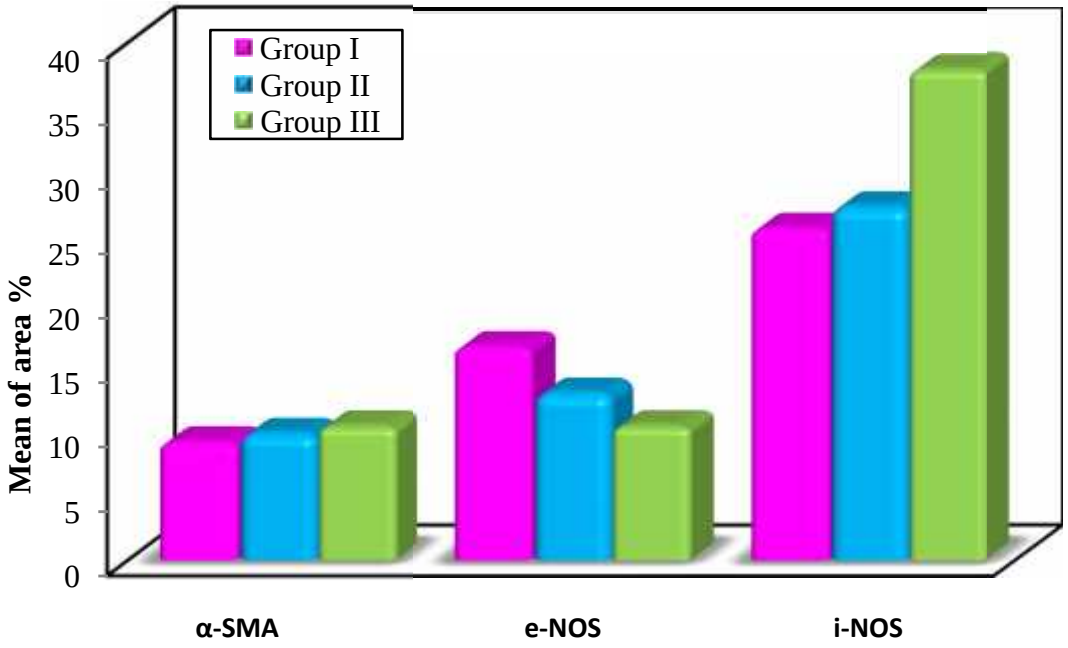


**Figure 8: Bar graph showing comparison between mean of area percentage of immunoreactions in different parameters of the three studied groups**

Ghareeb (2010) showed that ACR administration decreased the activities of glutathione (GSH), superoxide dismutase, and glutathione-S-transferase (GST). On the other hand, the activities of glutathione peroxidase (GPx) and catalase activities increased as a consequence of GSH depletion after ACR exposure. In supporting this study, Alturfan et al. (2012) and Lakshmi et al. (2012) showed that in ACR group, GSH levels also decreased significantly while the malondialdehyde levels, myeloperoxidase activity and collagen content increased in the tissues suggesting oxidative organ damage. Moreover, they reported increase in serum enzyme activities, cytokine levels and leukocyte late apoptosis**.**

Several studies in their explanations on the basis that acrylamide contained in potato chips caused oxidative stress by inducing the generation of ROS, reducing the antioxidant defense systems of cells via depleting non enzymatic antioxidant system (vitamins and glutathione) and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition (Lim et al., 2004 and Lee and Jacobs, 2005 and Jiang et al., 2007).

Our present results showed moderate SDH activity in kidney tubules after low dose of acrylamide in addition to diminished enzyme activity in group III treated with high dose of acrylamide compared with control group which showed strong activity. This current result is confirmed by data obtained from our image analysis study which revealed significant decrease in the mean of area percentage of SDH in groups II (22.26 ± 3.12) and III (19.77 ± 2.54) after acrylamide administration as compared with control group (29.38 ± 3.57).

Along with ubiquinone, SDH is a crucial antioxidant enzyme in mitochondria controlling superoxide scavenging activity of the respiratory chain. When succinate-ubiquinone activity is inhibited, electrons that would normally transfer through the SDH -B subunit to the ubiquinone pool are instead transferred to O2 directly to produce ROS, mainly superoxide anion (Rustin et al., 2002).

It is well known that proximal tubular cells contain large numbers of mitochondria and are the most reliant upon oxidative phosphorylation and most susceptible to oxidant-induced apoptosis and mutations (Bagnasco et al., 1985).

SDH activity is an indicator of mitochondrial activity and any decrease in its activity suggesting mitochondrial dysfunction (Abraham et al., 2013). Baysal (2006) declared that decreased SDH activity indicated loss of inner mitochondrial membrane integrity and the role of SDH was not only in mitochondrial energy generation, but also has a role in oxygen sensing. In accordance, the present result may be attributed to increased ROS production and hence oxidative stress suggesting damage of mitochondria which may contribute to depletion of SDH activity in acrylamide treated rat kidneys. In this context, Özturan-Özer et al. (2014) revealed that acrylamide treatment caused an oxidative stress in a tissue and ultrastructural modulations were especially detected in epithelial cells of proximal tubules in kidney induced as a result of high dose of acrylamide. In sight of those

findings, they suggested that proximal tubules were on the way of development of early tissue damage.

With respect to LDH, it is widely distributed throughout the body and is found at high levels in tissues that utilize glucose for energy; it is therefore not organ specific. As a result, an increase in LDH can reflect damage to a number of different tissues (skeletal or cardiac muscle, kidney, liver) (DuBose, 2008).

In our present results, LDH activity after acrylamide treatment revealed irregular distributed coarse granules with moderate activity after low dose and strong activity after high dose compared with control group which showed weak LDH activity. Activity of LDH recorded a significant increase in mean area percentage of group III (39.86 ± 7.06) as compared with group II (27.99 ± 3.77) and control (20.77 ± 5.27) at $p \leq 0.05$. This is an adaptive manner to keep energy production in order to maintain vital processes inside the cells after cellular injuries. These results support the study of Reddy et al. (2012), where the authors showed a significant ($p < 0.01$) reduction in the activities of oxidative enzymes succinate dehydrogenase (SDH), malate dehydrogenase (MDH), glutamate dehydrogenase (GDH) and isocitrate dehydrogenase (ICDH), while lactate dehydrogenase (LDH) activity was significantly ($p < 0.01$) increased in diabetic rats.

Xanthine oxidoreductase (XOR) enzyme occurs in most mammalian tissues, and although it has a broad specificity for reducing substrates, its conventionally accepted role is in purine catabolism, catalyzing the oxidation of hypoxanthine to xanthine and the oxidation of xanthine to uric acid. Despite XOR has wide tissue distribution, the enzyme is believed to be largely concentrated in endothelial and epithelial cells. (Martin et al., 2004). XOR has been implicated in the development of tissue oxidative damage in a wide variety of respiratory and cardiovascular disorders such as acute lung injury, ischemia reperfusion injury, atherosclerosis, heart failure, and arterial hypertension (Boueiz et al., 2008). Numerous inflammatory disorders are associated with elevated levels of xanthine oxidoreductase (XOR) and allied enhancement of reactive species formation contributory to systemic pathology (Cantu-Mdellin and Kelley, 2013). The reduction products of oxygen are reactive oxygen species; the main product of the xanthine oxidase reaction is $H_2O_2$ rather than $O^-_2$ (Berry and Hare, 2004).

The current study showed that XOR activity was observed with weak intensity in control group. After acrylamide administration, kidney sections revealed moderate and strong enzyme activity after low and high doses respectively. A significant increase in mean of area percentage of XOR activity after high dose of acrylamide (35.24 ± 6.51) as compared with low dose (23.15 ± 4.26) and control group (19.17 ± 3.76) at $p \leq 0.05$.

The increment in XOR activity could be due to the development of tissue oxidative damage that caused by ACR.

Regarding α-SMA, our results showed minimal proliferation in α-SMA expression in kidney sections of after acrylamide administration comparing with control. Mean of area percentage revealed no significant difference ($p > 0.05$) after low (9.79 ± 1.62) and high dose (10.33 ± 2.12) as compared with control (9.10 ± 1.54). These minor changes in α-SMA expression may be attributed to kidney injury induced by ACR rather than less extent nephropathy and these findings were in accord with many previous studies in experimental nephropathy.

The appearance of α -SMA myofibroblasts is not confined to animal models of nephropathy but its detection can be used as a morphological predictor of progressive disease (Pilmore et al., 2000 and . Mezzano et al., 2000). In an experimental model of progressive nephropathy, Abbate et al. (2002) indicated that proximal tubular cells promote peritubular accumulation of α-SMA+ cells, either directly and via macrophage mediated pathways. In a remnant kidney model, investigators revealed that kidneys from 5/6 subtotal nephrectomy hypertensive rats showed a 10-fold increase in α -SMA staining compared to controls (Nutter et al., 2012). In the kidney tubulointerstitial expression of α-SMA, a microfilament that marks epithelial-mesenchymal transition and fibroblast activation, increased significantly as a result of cyclosporine A -induced nephrotoxicity (Carlos et al., 2014).

NO derived from NOS plays an important role in renal function, both under normal and pathophysiological conditions ( Shoskes & Halloran, 1996 and Nath and Norby, 2000). NO increases renal injury through its reaction with superoxide radical and generation of a cytotoxic peroxynitrite which damaged the tubular cells (Walker, et al., 2000). Superoxide and NO can react to the powerful oxidant and nitrating agent peroxynitrite ($ONOO^-$) and reducing the bioavailability of NO and thus resulting in vascular dysfunction. (Feletou and Vanhoutte , 2006)]

ACR administration increased the lipid peroxidation and NO levels of both liver and brain tissues (Ghareeb, 2010). ACR administered rats showed increased levels of lipid peroxidative product, protein carbonyl content, hydroxyl radical and hydroperoxide which were significantly modulated by the supplementation of fish oil (Lakshmi et al., 2012).

The current result revealed moderate e-NOS immunoreactivity in cytoplasm of most proximal and distal tubules of control sections and weak positivity of e-NOS expression was recorded in tubular cytoplasm of kidney sections in group II along with group III after acrylamide administration. The immunoreactivity of i-NOS showed moderate expression in control group and group II while group III recorded strong expression of i-NOS. Moreover, the mean area percentage declared statistically significant lower immunoreaction ($p \leq 0.05$) of e-NOS in group II (12.97 ± 2.40) and group III (10.40 ± 1.22) as compared with group I (16.54 ± 2.11). Higher significant difference ($p \leq 0.05$) of i-NOS was observed in group III (38.03 ± 6.89) as compared with each of group I (25.72 ± 4.24) and group II (27.41 ± 5.73).

Collectively, these current results were in accord with Furusu et al., (1998) who found that the extent of eNOS expression is negatively correlated with the degree of glomerular injury, while the extent of iNOS expression is positively correlated with the degree of glomerular injury in the same tissues. Moreover, transient eNOS-mediated NO production is essential for vasorelaxation, antiapoptosis, and protection against oxidative stress while sustained iNOS-mediated NO generation may mediate lipid peroxidation, DNA damage, and proapoptotic effects (Förstermann and Sessa, 2012).

Sadek (2012) reported that acrylamide caused many adverse effects in the tissues reflected in significant increase in lipid peroxidation, decrease in glutathione levels and decreased activities of catalase and superoxide dismutase.

So in conclusion: the present results showed that the two doses of acrylamide exerted deleterious effects on some enzyme activities and on immunohistochemical expressions of some parameters in a dose-dependent manner.

## 5 References


Abbate, M., Zoja C., Rottoli, D., Corna, D., Tomasoni, S., Remuzzi, G. (2002). Proximal tubular cells promote fibrogenesis byTGF-β1–mediated induction of peritubular myofibroblasts. Kidney International, 61 : 2066–2077.

Abraham, P., Ramamoorthy, H., Isaac, B. (2013).Depletion of the cellular antioxidant system contributes to tenofovir disoproxil fumarate - induced mitochondrial damage and increased oxido-nitrosative stress in the kidney. Journal of Biomedical Science, 20(61): 1-15

Alturfan, A.A., Tozan-Beceren, A., Sehirli, A.O., Demiralp, E., Sener, G., Omurtag, G.Z. (2012). Resveratrol ameliorates oxidative DNA damage and protects against acrylamide-induced oxidative stress in rats. Mol Biol Rep., 39(4):4589-4596.

Bagnasco, S., Good, D., Balaban, R., Burg, M. (1985).Lactate production in isolated segments of the rat nephron. Am J Physiol., 248:F522–F526.

Balaban, R. S., Nemoto, S., Finkel, T. (2005). Mitochondria, oxidants, and aging. Cell, 120(4): 483–495

Baysal, B.E. (2006). A phenotypic perspective on Mammalian oxygen sensor

candidates. Ann NY Acad Sci., 1073:221–233.

Berry, C. E., Hare, J.M. (2004). Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol., 555: 589– 606.

Boueiz, A., Damarla, M., Hassoun, P. M.( 2008). Xanthine oxidoreductase in respiratory and cardiovascular disorders. Am J Physiol Lung Cell Mol Physiol., 294(5): L830–L840

Burdmann, E.A., Andoh, T.F., Yu, L., Bennett, W.M. (2003). Cyclosporine nephrotoxicity. Semin Nephrol., 23: 465–476

Cantu-Medellin, N., Kelley, E.E. (2013). Xanthine oxidoreductase-catalyzed reduction of nitrite to nitric oxide: Insights regarding where, when and how. Nitric Oxide, 34:19-26.

Carlos, C. P., Sonehara, N. M., Oliani, S. M., Burdmann, E.A. (2014). Predictive Usefulness of Urinary Biomarkers for the Identification of Cyclosporine A-Induced Nephrotoxicity in a Rat Model. PLoS One, 9(7): e103660.

Cherng, S., Young, J., Ma, H. (2008). Alpha-Smooth Muscle Actin (α-SMA). The Journal of American Science, 4(4): 7-9.

DuBose, T.D. Jr. (2008). Disorders of acid-base balance. In: Brenner BM, ed. Brenner and Rector's. The Kidney. 8th ed. Philadelphia, Pa: Saunders Elsevier; chap 14.

Feletou, M., Vanhoutte, P.M. (2006). Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). Am J Physiol Heart Circ Physiol., 291:H985–H1002.

Förstermann, U., and. Sessa, W. C. (2012). "Nitric oxide synthases: regulation and function," Eur Heart J., 33(7):829-837.

Frederiks, W.M., Marx, F. (1993). A histochemical procedure for light microscopic demonstration of xanthine oxidase activity in unfixed cryostat sections using cerium ions and a semipermeable membrane technique. J Histochem Cytochem., 41(5):667-670.

Furusu, A., Miyazaki, M. , Abe ,K. , Tsukasaki, S., Shioshita, K. , Sasaki, O. , Miyazaki , K. , Ozono, Y. , Koji, T. , Harada ,T., Sakai, H., Kohno, S.( 1998). "Expression of endothelial and inducible nitric oxide synthase in human glomerulonephritis," Kidney International, 53(6):1760–1768.

George, K. S., Narayanareddy, R., Muchukunte, M. S. B., Muralidhara. (2013). Prophylaxis with Bacopa monnieri Attenuates Acrylamide Induced Neurotoxicity and Oxidative Damage via Elevated Antioxidant Function. Central Nervous System Agents in Medicinal Chemistry, 13(1): 3-12.

Ghareeb, D.A.( 2010). Ameliorated effects of garlic (Allium sativum) on biomarkers of subchronic acrylamide hepatotoxicity and brain toxicity in rats . Toxicological and Environmental Chemistry, 92:1357-1372.

Goto, S., Yamamoto, T., Feng, L., Yaoita, E. , Hirose, S., Fujinaka, H.( 1995) . Expression and localization of inducible nitric oxide synthase in anti-Thy-1 glomerulonephritis. Am J Pathol., 147(4):1133-1141.

Hassan, S. M., (2005). Effect of acrylamide on albino rat kidney: histological, histochemical and electron microscopy studies. Bull Alex Fac Med., 41(2): 91-100.

Hellberg, C.B., Boggs, S.E., Lapetina, E.G. (1998). Phosphatidylinositol 3-kinase is a target for protein tyrosine nitration. Biochem Biophys Res Commun., 252(2) :313–317.

HUANG, S. , MILLAR, A. H. (2013). SUCCINATE DEHYDROGENASE: THE COMPLEX ROLES OF A SIMPLE ENZYME. CURR OPIN PLANT BIOL., 16(3):344-349.

Jiang, L. , Cao, J., An, Y., Geng, C., Qu, S., Zhong, L.( 2007). Genotoxicity of acrylamide in human hepatoma G2 (HepG2) cells. Toxicol In Vitro: 21:1486–1492.

Kavanagh, K.L., Elling, R.A.,Wilson, D.K. (2004) . Structure of *Toxoplasma gondii* LDH1: active-site differences from human lactate dehydrogenases and the structural basis for efficient APAD+ use. *Biochem.* 43(4): 879–889.

Kooij, A., Frederiks, W.M., Gossrau, R., Van Noorden, C.J.F.(1991). Localization of xanthine oxidoreductase activity using the tissue protectant polyvinyl alcohol and final electron acceptor tetranitro BT. J Histochem Cytochem., 39: 87-93.

Krishnakumar, T., Visvanathan, R. (2014) Acrylamide in Food Products: A Review. J Food Process Technol., 5: 344. doi:10.4172/2157- 7110.1000344 pages 1-9

Lakshmi, D., Gopinath, K. , Jayanthy , G. , Anjum, S., Prakash, D., Sudhandiran, G.(2012) Ameliorating Effect of Fish Oil on Acrylamide Induced Oxidative Stress and Neuronal Apoptosis in Cerebral Cortex. Neurochemical Research, 37: 1859-1867.

Lamas, S., Marsden, P.A., Li, G.K. , Tempst, P., Michel, T.(1992). Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform. Proc Natl Acad Sci U S A, 89(14): 6348-6352.

Lee, D.H., Jacobs, D.R. Jr. (2005): "Association between serum gamma-glutamyltransferase and C-reactive protein." Atherosclerosis, 178: 327-330.

Lim, J.S., Yang, J.H. Chun, B.Y., Kam, S., Jacobs, D.R. Jr., Lee, D.H. (2004): "Is serum gamma-glutamyltransferase inversely associated with serum antioxidants as a marker of oxidative stress?. Free Radic.Biol. Med., 37: 1018-1023.

Martin, H.M., Hancock, J.T., Salisbury, V., Harrison, R.( 2004). Role of Xanthine Oxidoreductase as an Antimicrobial Agent. Infect Immun., 72(9):4933-4939.

McDonald, M.C., Mota-Filipe, H., Paul, A., Cuzzocrea, S., Abdelrahman, M.(2001). Calpain inhibitor I reduces the activation of nuclear factor-_B and organ injury/ dysfunction in hemorrhagic shock. FASEB J., 15:171–186.

Mezzano, S.A., Droguett, M.A., Burgos, M.E., Ardiles, L.G., Aros, C.A., Caorsi, I., Egido, J.(2000). Overexpression of chemokines, fibrogenic cytokines, of chemokines, fibrogenic cytokines, and myofibroblast in human membranous nephropathy. Kidney Int. 57(1):147–158.

Micili, S.C., Goker, A., Sayin, O., Akokay, P., Ergur, B.U.( 2013). The effect of lipoic acid on wound healing in a full thickness uterine injury model in rats. J Mol Histol., 44(3): 339-345.

Moncada, S., Higgs, A. (1993). The L-arginine-nitric oxide pathway. N Engl J Med., 329(27):2002–2013.

*Morsy, M.* A., *Ibrahim, S.* A., *Amin, E. F.*, *Kamel M. Y.*, *Rifaai R.* A., *Hassan M. K.* (2014). Sildenafil Ameliorates Gentamicin-Induced Nephrotoxicity in Rats: Role of iNOS and eNOS. Journal of Toxicology, 2014: 1-7.

Murphy, M.P. (2009). How mitochondria produce reactive oxygen species. Biochem J. , 417:1–13.

Nakatani, T., Nakashima, T., Kita, T. (1999) . Succinate dehydrogenase activities of fibers in the rat extensor digitorum longus, soleus, and cardiac muscles. Arch Histol Cytol., 62:393–399.

Nath, K.A., Norby, S.M.( 2000) Reactive oxygen species and acute renal failure. Am J Med., 109(8):655–678.

Nutter, F., Khwaja, A., Haylor, J. (2012). Seliciclib inhibits renal hypertrophy but not fibrosis in the rat following subtotal nephrectomy. Nephron Exp Nephrol., 122: 114-122.

Ozbek, E. (2012). Induction of Oxidative Stress in Kidney. Int J Nephrol. , 2012:465897. doi: 10.1155/2012/465897. pages 1-9

Özturan -Özer, E., Uçar, G., Helvacıoğlu, F., Akaydın -Aldemir, D., Türkoğlu, S.*(2014).* Effect of acrylamide treatment on arginase activities and nitric oxide levels in rat liver and kidney. Acta Medica Mediterranea, 30: 375-382.

Pearse, A.G.E. (1972).Histochemistry Theoritical and Applied 3rd Edition. London, New York: Churchill Living stone, 2: 1342-1344.

Pilmore, H.L., Painter, D.M., Bishop, G.A., Mc Caughan, G.W., Eris, J.M. (2000): Early up-regulation of macrophages and myofibroblasts. Transplantation, 69(12) :2658–2669.

Radermacher, J., Klanke, B., Schurek, H.J., Stolte, H. F., Frolich, J. C.(1992) Importance of NO/EDRF for glomerular and tubular function: studies in the isolated perfused rat kidney. Kidney International, 41(6) : 1549–1559.

Reddy, S. R. , Lavanya, T. , Narasimhulua, G., Reddy, K. S.(2012). Effect of Pimpinella tirupatiensis on Oxidative Enzymes in STZ-induced Diabetic Rat Kidney. Iranian Journal of Pharmaceutical Research, 11 (1): 277-286.

Rustin, P., Munnich, A., Rötig, A. (2002). Succinate dehydrogenase and human diseases: new insights into a well-known enzyme. Eur J Hum Genet., 10:289–291

Sadek, K.M. (2012). Antioxidant and immunostimilant effect of carica papaya linn. Aqueous extract in acrylamide intoxicated rats. Acta Inform Med., 20(3): 180–185.

Shoskes, D.A., Halloran, P.F. (1996). Delayed graft function in renal transplantation: Etiology, management and long-term significance. J Urol., 155:1837–1840.

Skalli, O., Ropraz, P., Trezeciak, A., Benzonana, G., Gillessen, D., Gabbiani, G._(1986). A monoclonal antibody against alpha-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol., 103:2787-2796.

Tabouy, L. , Zamora, A.J., Oliva, L. , Montet, A.M., Beauge, F. , Montet, J.C. (1998) Ursodeoxycholate protects against ethanol-induced liver mitochondrial injury. Life Sci. 63: 2259–2270.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Törnqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. Journal of Agricultural and Food Chemistry, 50: 4998-5006.

Teodor , V., Cuciureanu, M., Filip, C., Zamosteanu, N., Cuciureanu, R.( 2011). Protective effects of selenium on acrylamide toxicity in the liver of the rat. Effects on the oxidative stress. Rev. Med. Chir. Soc. Med. Nat. Iasi., 115 (2): 612-618.

Thiemermann, C.T., Ruetten, H., Wu, C.C., Vane, J.R. (1995). The multiple organ dysfunction syndrome caused by endotoxin in the rat: attenuation of liver dysfunction by inhibitors of nitric oxide synthase. Br J Pharmacol., 116(7):2845–2851.

Walker, L. M.,Walker, P. D., Imam, S. Z. , Ali, S. F., Mayeux, P. R.(2000).Evidence for peroxynitrite formation in renal ischemia-reperfusion injury: studies with the inducible nitric oxide synthase inhibitor L-N6-(1-iminoethyl) lysine. J Pharmacol Exp Ther., 295(1): 417-422.

Yokozawa, T., Chung, H.Y., Kim, D.W. Goto, H. (1999). Involvement of superoxide and/or nitric oxide in renal tissue injury. Exp Toxicol Pathol. , 51: 517–521.

Zhang, Q. J., Mc Millin, S.L., Tanner, J.M., Palionyte, M., Abel, E.D., Symons, J.D. (2009). Endothelial nitric oxide synthase phosphorylation in treadmill-running mice: role of vascular signalling kinases. J Physiol., 587:3911–3920.