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The combined protective effect of N-acetylcysteine and vitamin E against acrylamide-induced nephrotoxicity in rats.

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ABSTRACT

The purpose of the present study to evaluate the antioxidant and nephroprotective effects of N-acetylcysteine (NAC) and/or Vitamin E (Vit E) against renal damage induced by acrylamide (ACR) in rats. The antioxidant and nephroprotective efficacy of NAC and Vit E against ACR-induced nephrotoxicity in rats was investigated in this work. The rats were assigned to equal seven groups (n=7): control vehicle group (saline only), NAC group (150 mg/kg, orally), Vit E group (100 mg/kg, orally), ACR group (20 mg/kg, orally), NAC+ACR group, Vit E+ACR group, and NAC+Vit E+ACR group and all treatments were given for 30 days. Creatinine, urea, and glucose concentrations in the blood were increased as a result of ACR administration. Furthermore, ACR reduced protein and albumin levels. Malondialdehyde levels in kidney tissues were increased dramatically and reduced glutathione, superoxide dismutase, and catalase levels were decreased. ACR also caused certain degenerative lesions in the kidney tissues and elevated caspase-3 expression. On conclusion: administration of NAC and Vit E alone or in combinations ameliorated the renal toxicity and apoptosis induced by ACR.

1. INTRODUCTION

The kidney is a soft organ that is responsible for the removal of potentially hazardous materials from the body as well as the formation of urine (Elsayed et al., 2014; Abugomaa and Elkomy et al., 2019; Elbadawy, 2020; Soliman et al., 2022 a,b). The liquid electrolyte balance in the body is also regulated by the kidneys (Erdemli et al., 2017).

Acrylamide (ACR) is a crystalline monomer that is odorless and colorless and has a high chemical reactivity (Acaroz et al., 2018). It's a water-soluble molecule used in a variety of applications, including soil coagulation, color synthesis, paper packing, laboratory reasons, and wastewater treatment (Elblehi et al., 2020). As a result of the excessive generation of reactive oxygen species (ROS), ACR exposure induces an imbalance in oxidant and antioxidant levels, which plays a critical role in ACR-induced toxicity (Abdel-Daim et al., 2015; Song et al., 2013). In previous studies, ACR was associated with nephrotoxicity (Ghorbel et al., 2017; Elhelaly et al., 2019).

N-acetylcysteine (NAC) is an amino acid that contains sulphur with an effective antioxidant. It's a more stable form of the amino acid L-cysteine, which is a source of cysteine required for the production of glutathione, a major nonenzymatic intracellular antioxidant (Tenório et al., 2021). Vitamin E (Vit E) is a lipid-soluble substance essential to the cell's defensive system. Because of its antioxidant properties, it has a strong preventive effect against the consequences of many diseases (Böhm 2018). Vitamin E can be found in a variety of foods, including seeds, vegetable oils, and nuts. Vitamin E provides a long list of health benefits against heart disease, cancer, and musculoskeletal ailments (Chin and Ima-Nirwana, 2019). It can be used to considerably reduce the kidney damage caused by oxidative stress and the formation of ROS. Several animal studies have shown that vitamin E has nephroprotective effects against toxic substances including colistin (Ghlissi et al., 2018) and aflatoxin (Abdel-Hamid and Firgany 2015).

The antioxidant and nephroprotective efficacy of NAC and Vit E against ACR-induced nephrotoxicity in rats was investigated in this work.

2. MATERIAL AND METHODS

2.1. Chemicals:

ACR is a white powder (Sigma-Aldrich, USA). NAC was obtained from SEDICO (6 October City, Egypt). Pharco Pharmaceuticals Industries (Alexandria, Egypt) provided vitamin E. The analytical kits were provided by Bio-diagnostics Company (Giza, Egypt).

2.2. Experimental design:

Forty-nine Wister Albino male rats with a weight of 185±20 g was obtained from The Egyptian Organization for Biological Products and Vaccines. All animals were

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acclimatized for 7 days at a temperature of 25±2˚C, with a 12:12 h light/dark cycle and free access to water and commercial pellets. Rats were randomly assigned to seven groups: control group (saline, once daily, PO); NAC group (150 mg/kg NAC, once daily, PO (Elsayed et al., 2021); Vit E group (100 mg/kg Vit E once daily, PO (Aboubakr et al., 2020); ACR toxic control group administered 20 mg/kg ACR orally once daily (Elkomy et al., 2018; Aboubakr et al., 2019); the NAC+ACR, Vit E+ACR, and NAC+Vit E+ACR groups received NAC, Vit E, and/or ACR as described above. All treatments were administered for 30 days. Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Benha University approved the experimental protocol (BUFVTM, 10-03-22).

2.3. Sampling and processing:

One day following the last treatment, Isoflurane was used to anaesthetize rats. The serum was separated using blood samples taken from the retro-orbital plexus (centrifugation at 1200 g for 15 min). For subsequent biochemical examination, the separated sera were kept at -20˚C. Creatinine, urea, glucose, total protein, and albumin levels were estimated.

The kidneys were rapidly removed, cleaned in saline, and perfused with ice-cold sodium phosphate-buffered saline (100 mmol/L Na2HPO4/NaH2PO4, pH 7.4) containing 0.1 mmol/L EDTA (Abdel-Daim et al., 2020). The tissue samples were kept at -80°C. The tissue samples were homogenised on ice in an electrical homogenizer with 5 ml phosphate buffer pH 7.4 and 1g tissue. To avoid GSH oxidation, N-ethylmaleimide was introduced immediately after homogenization. The homogenates were centrifuged at 1200 x g for 20 minutes at 4ºC to separate the supernatants after homogenization. The indicators of oxidative stress were detected using these supernatants. Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) were the oxidative stress indicators that were assessed.

2.4. Histopathology:

The kidneys of control and experimental rats were carefully removed, fixed in 10% neutral buffered formalin. Then dehydrate in ascending strength of ethyl alcohol, cleaned in xylene, and then embedded in paraffin wax. 5 m paraffin slices were cut and stained with hematoxylin and eosin (H&E) staining according to Bancroft and Gamble (2008).

2.5. Caspase-3 immunohistochemistry localization:

Kidney sections at 5 µm from each rat were deparaffinized, rehydrated, and incubated in 3% H_2O_2 for 10 minutes. The sections were then treated at room temperature for 1 hour with rabbit monoclonal antibody anti-caspase-3 (Santa Cruz Biotechnology Inc., Dallas, TX, USA, 1:100 dilutions). For 30 minutes, the sections were incubated with goat anti-rabbit IgG. For 10 minutes, diaminobenzidine (DAB) was used to visualize the immunoreaction (Aboubakr et al., 2021).

2.6. Statistical analysis:

The recorded data were expressed as mean + standard error. Data were analyzed using one-way ANOVA followed by Duncan's post hoc test for multiple group comparisons using the statistical software package SPSS for Windows (Version 21.0; SPSS Inc., Chicago, IL, USA). At $P \leq 0.05$, differences were judged statistically significant.

3. RESULTS

ACR induced nephrotoxicity evidenced by higher serum levels of renal biomarkers (Table 1). When compared to control rats, the concentrations of creatinine, urea, and glucose were significantly higher after ACR intoxication. ACR also lowered total protein and albumin concentrations in the blood. On the other hand, when compared to the ACR group, these parameters were dramatically decreased in the ACR-intoxicated rats given NAC, Vit E, or a combination (NAC and Vit E). When ACR-intoxicated rats were treated with both NAC and Vit E, these parameters were shifted towards control levels compared to treatment with either NAC or Vit E alone.

Table 2 shows the effects of ACR intoxication and treatment with NAC, Vit E, or a combination on MDA, reduced glutathione, and antioxidant enzymes in kidney tissues. In ACR-intoxicated rats, MDA levels increased considerably, while CAT, SOD, and GSH levels were declined dramatically. The effects of ACR on MDA in kidney tissues, SOD, CAT, and GSH were reduced by NAC and Vit E, but these values have remained significantly different from control values. When compared to NAC or Vit E therapies alone, combined NAC and Vit E treatment dramatically reduced oxidative damage produced by ACR in renal tissues.

The microscopic examination of the kidneys in rats from the control, NAC, and Vit E groups; revealed normal renal histoarchitecture (Figs 1A-C). Atrophy of the glomeruli with widened Bowman's space (Figs 1D,E), severe congestion and dilatation of interstitial blood vessels (Figs 3D-F), vacuolated renal tubular epithelium (Fig 1E), and cellular debris (Fig 1F) in the lumen of renal tubules were the main the histopathological lesions seen in the ACR-treated group Although kidney sections from ACR+NAC, ACR+Vit E, and ACR+NAC+Vit E treated rats showed fewer apparent histological abnormalities than ACR-exposed rats, the ACR+NAC+Vit E group demonstrated the best protection, with normal glomeruli, renal tubules, and interstitial blood vessels (Figs 2A-C).

The immunohistochemistry expression of caspase3 in the cytoplasm of renal tissue cells was much stronger in the ACR group (Fig.3D), compared to significantly weak caspase3 immunoreactivity in renal sections from the control, NAC, and Vit E groups (Figs.3A-C). When compared to the ACR group, the ACR+NAC, ACR+Vit E and ACR+NAC+Vit E groups showed mild immunoreaction (Figs. 3E, F, G).

Table 1 Effects of NAC, Vit E and/or ACR on serum creatinine, urea, glucose, totl protein and albumin in control and treated groups (n=7).

Parameters	Control	NAC	Vit E	ACR	ACR + NAC	ACR + Vit E	ACR+NAC + Vit E
Creatinine (mg/dl)	0.80 ± 0.01^{d}	0.78 ± 0.02^{d}	0.79 ± 0.01^{d}	1.83± 0.02^{a}	1.05± 0.03^{b}	1.09± 0.04^{b}	0.91± 0.02^{c}
Urea (mg/dl)	30.61± 1.06^{e}	31.10± 1.20^{e}	29.92± 1.04^{e}	95.50 ± 1.26^{a}	72.91 ± 1.15^{b}	63.27 ± 1.06^{c}	40.40 ± 1.25^{d}
Glucose (mg/dl)	122.32± 1.46^{e}	126.28± 3.09^{e}	124.38± 1.95^{e}	278.05± 6.05^{a}	228.48± 4.73^{b}	198.79± 4.46^{c}	149.62± 2.54^{d}
T. Protein (g/dl)	8.15 ± 0.05^{a}	8.07 ± 0.05^{a}	8.11 ± 0.04^{a}	6.01± 0.04^{e}	6.78± 0.17^{d}	7.16± 0.04^{c}	7.49± 0.13^{b}
Albumin (g/dl)	4.15 ± 0.03^{a}	4.18 ± 0.02^{a}	4.12 ± 0.01^{a}	2.99± 0.05^{c}	3.46± 0.07^{b}	3.59± 0.13^{b}	4.08± 0.06^{a}

Data are expressed as the mean ± SE. Different superscript letters in the same row indicate statistical significance at $P \leq 0.05$.
Acrylamide (ACR); N-acetylcysteine (NAC); Vitamin E (Vit E).

Table 2 Effects of NAC, Vit E and/or ACR on antioxidant parameters in renal tissues (n=7).

Parameters	Control	NAC	Vit E	ACR	ACR+ NAC	ACR+ Vit E	ACR+NAC + Vit E
MDA (nmol/)	86.35± 2.55^{d}	89.33± 2.68^{d}	86.99± 1.42^{d}	163.07± 4.36^{a}	130.17± 2.53^{b}	135.81 ± 4.90^{b}	106.82± 2.24^{c}
CAT (U/g)	2.78± 0.05^{a}	2.84± 0.02^{a}	2.81± 0.04^{a}	1.22± 0.03^{d}	1.95± 0.02^{c}	1.97± 0.03^{c}	2.36± 0.07^{b}
SOD (U/g)	18.24± 0.29^{a}	18.65± 0.33^{a}	17.88± 0.45^{a}	5.93± 0.32^{d}	7.17± 0.19^{c}	7.98± 0.43^{bc}	8.78± 0.33^{b}
GSH (mg/g)	92.39± 2.04^{a}	91.78± 2.75^{a}	89.69± 1.09^{a}	57.01± 0.94^{e}	65.05± 2.13^{d}	72.72± 2.48^{c}	81.36± 4.11^{b}

Data are expressed as the mean ± SE. Different superscript letters in the same row indicate statistical significance at $P \leq 0.05$.
Acrylamide (ACR); N-acetylcysteine (NAC); Vitamin E (Vit E); Malondialdehyde (MDA); catalase (CAT); superoxide dismutase (SOD); reduced glutathione (GSH).

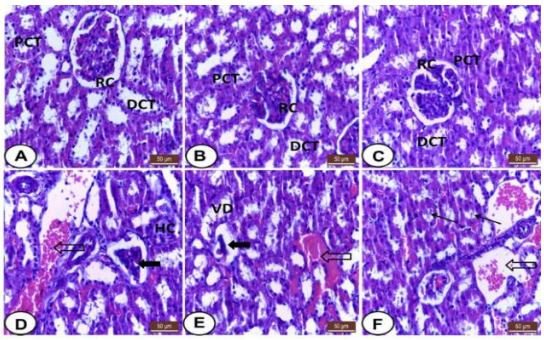

Figure 1 Histological sections of kidneys from Control, NAC, Vit E and ACR groups. A, B and C; Control, NAC and Vit E groups showed normal renal histo-architecture; renal corpuscle (RC), proximal (PCT) and distal tubules (DCT). D-F; ACR intoxicated rats showed shrinkage of the glomeruli with widened Bowman's space (black arrows), severe congestion and dilatation of interstitial blood vessels (hollow arrows), vacuolated renal tubular epithelium (VD), and cellular debris (thin arrows) in the lumen of renal tubules. H&E stain, scale bars=50µm.

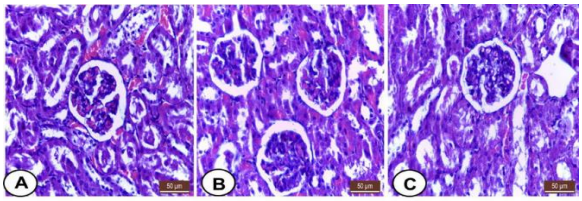

Figure 2 Histological sections of kidneys from NAC+ACR, Vit E+ACR and NAC+Vit E+ACR groups. A-B; renal sections from ACR+NAC and ACR+Vit E treated groups showed less prominent histopathological alterations compared to ACR-exposed rats. C; ACR+NAC+Vit E groups exhibited the best protection that was noted in the form of normal glomeruli, renal tubules and interstitial blood vessels. H&E stain, scale bars=50µm.

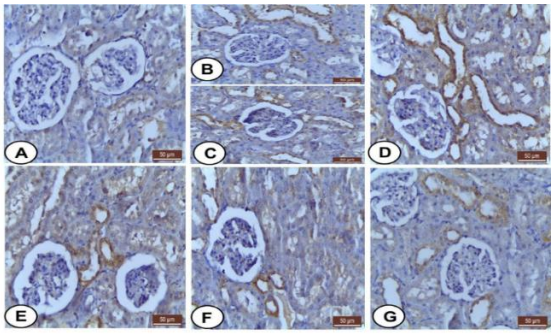

Figure 3 Immunohistochemical staining of caspase3 in renal sections from all examined groups. A, B and C; Control, NAC and Vit E groups showed weak renal caspase3 immunoreaction. D; ACR treated group revealed strong expression of caspase3 in renal tissues. E-F; NAC+ACR and Vit E+ACR treated rats showed moderate expression of caspase3 compared with CP group. G; NAC+Vit E+ACR group showed weak caspase3 immunoreaction. Scale bars = 50µm.

4. DISCUSSION

Acrylamide is a major public health issue in the environment. ACR was studied as a possible kidney toxin (Rajeh and Al-Dhaheri, 2017). Creatinine and BUN are nitrogenous nonprotein components that result from protein and nucleic acid degradation. In ACR-induced oxidative kidney injury, with high creatinine and BUN levels (Ghorbel et al., 2016). ACR intoxication may have harmed the brush border epithelial cells, rendering them impervious to urea and creatinine, resulting in their high blood levels (Uthra et al., 2017). ACR increased creatinine and urea levels, which is consistent with other previous studies (Abdel-Daim et al., 2015; Sengul et al., 2021). ACR treatment resulted in considerable reductions in protein and albumin levels (Rivadeneyra-Domnguez et al., 2018). ACR intoxication lowers protein synthesis and changes the functional integrity of the kidney after hepato-renal injury, resulting in proteinuria and, eventually, lower circulating protein levels. ACR also raised blood glucose levels (Yue et al., 2020).

MDA is one of the end products of lipid peroxidation (LPO), which is linked to ROS, and it was shown to be considerably higher in the renal tissues of rats given ACR. It was postulated that oxidative stress might increase if the equilibrium between ROS generation and antioxidant capacity shifts in favor of the oxidants (Abdel-Daim et al., 2015). In the present study, renal MDA levels were elevated in ACR-treated rats, while GSH, SOD, and CAT activities were dramatically reduced. It has previously been shown that ACR detoxification conjugates with GSH, resulting in GSH depletion in the cells and an overabundance of free radicals and superoxide in the cells (Bin-Jumah et al., 2021). Multiple pathological diseases that were caused by oxidative stress, disrupt cellular function. These results are in agreement with Abdel-Daim et al., (2015); Ghorbel et al., (2017); Elhelaly et al., (2019); Bin-Jumah et al., (2021).

Scavenging free radicals, regulating immunity, antiapoptosis, and antioxidation are all roles of N-acetylcysteine, which is a precursor of intracellular glutathione (Elsayed et al., 2022a; Elsayed et al., 2022b; Elsayed et al., 2021). NAC may protect the kidneys from toxins by maintaining cell membrane integrity and enzyme activity (Yalçn and Gürel, 2021). NAC was found to reduce cisplatin-induced nephrotoxicity and restore normal kidney function in a previous investigation (Elsayed et al., 2021). This was demonstrated by the return of serum creatinine, urea, glucose, and protein levels to levels close to the control value. Furthermore, in renal tissue, the activity of the key enzymatic antioxidants was significantly increased (Abdel-Wahab et al., 2017).

The present study showed that pretreatment with NAC lowered MAD levels while increasing antioxidant enzymes SOD, GSH, and catalase levels. Previous studies mentined that the normal structure of interstitial tissue in the cortex, renal tubules, renal glomeruli, and collecting ducts in the medulla was revealed in NAC-treated rats (Abdel-Daim et al., 2019; Fan et al., 2020). NAC therapy reversed the elevated caspase-3 immunoreactivity, which is consistent with previous results of Siddarth et al., (2018).

Vitamin E is a lipid-soluble antioxidant that protects cell membranes and lipoproteins from lipid peroxidation by interrupting the lipid radical chain reaction (Böhm 2018). Several animal studies have revealed nephroprotective effects of vitamin E against toxic drugs such as colistin (Ghlissi et al., 2014), aflatoxin (Abdel-Hamid and Firgany,

2015), and vancomycin (Elyasi et al., 2013). Because of the antioxidant impact, oral treatment of vitamin E in rats resulted in considerable reductions in serum creatinine and urea levels (Abdel-Daim and Abdeen, 2018). The injection of vitamin E resulted in a statistically significant reduction in MDA levels (Mansour et al., 2014). Previous research concluded that vitamin E prevented the generation of reactive oxygen species (ROS), decreased lipid peroxide levels, increased glutathione (GSH), and increased superoxide dismutase and catalase activity (Abo-Elmaaty et al., 2020).

ACR intoxication was associated with glomeruli shrinkage, expanded Bowman's space, renal tubule degeneration, congestion of the interstitial blood vessels, and hyaline cast within the renal tubules in the kidney. All of these characteristics were found in El Fakahany et al. (2021) and Sengul et al (2021). The disruption of cell membrane integrity and fluidity caused by ACR may be responsible for this renal damage (Ghorbel et al., 2017). Vit E and/or NAC supplementation improved kidney histopathology findings in the current investigation. This could be due to their antioxidant qualities, as nitric oxide and glutathione are produced, which remove ROS and restore cellular equilibrium (Rajeh and Al-Dhaheri, 2017).

Regarding to ACR exposure, the results of immunohistochemical study revealed that marked immunoreaction of caspase 3 compared to the control group, which was consistent with previous study (El Fakahany et al., 2021). These could be due to ACR-induced ROS formation, lipid peroxidation, and GSH depletion, which led to the oxidative stress hypothesis, a decrease in lysosomal membrane localization, and oxidative stress-induced damage to mitochondrial and lysosomal membranes, which led to caspase-3 activation and cell death (Pedrycz et al., 2010). On other hand combining NAC and/or Vitamin E with ACR reduced caspase3 immunoreactivity.

5. CONCLUSION

Acrylamide induced considerable renal damage as evidenced by changed biochemical markers and histological abnormalities, due to oxidative stress and apoptotic processes. The combination of NAC and Vitamin E has been shown to protect the kidneys from ACR-mediated injury. Furthermore, both separately and in combination, NAC and Vitamin E offered significant kidney protection against ACR-induced oxidative stress and apoptosis.

6. REFERENCES

1. Abdel-Daim MM, Abd Eldaim MA, Hassan AG (2015) *Trigonella foenum-graecum* ameliorates acrylamide-induced toxicity in rats: Roles of oxidative stress, proinflammatory cytokines, and DNA damage. Biochem Cell Biol. 93(3):192-198.
2. Abdel-Daim MM, Abdeen A (2018) Protective effects of rosuvastatin and vitamin E against fipronil-mediated oxidative damage and apoptosis in rat liver and kidney. Food Chem Toxicol. 114:69-77.
3. Abdel-Daim MM, Dawood MAO, Elbadawy M, Aleya L, Alkahtani S. (2020) Spirulina platensis Reduced Oxidative Damage Induced by Chlorpyrifos Toxicity in Nile Tilapia (Oreochromis niloticus). Animals (Basel). Mar 12;10(3):473. doi: 10.3390/ani10030473.
4. Abdel-Daim MM, Dessouki AA, Abdel-Rahman HG, Eltaysh R, Alkahtani S. (2019) Hepatorenal protective effects of taurine and N-acetylcysteine against fipronil-induced injuries: The antioxidant status and apoptotic markers expression in rats. Sci Total Environ. 650(Pt 2):2063-2073.
5. Abdel-Hamid AA, Firgany Ael-D. (2015) Vitamin E supplementation ameliorates aflatoxin B1-induced nephrotoxicity in rats. Acta Histochem. 117(8):767-779.
6. Abdel-Wahab WM, Moussa FI, Saad NA. (2017) Synergistic protective effect of *N*-acetylcysteine and taurine against cisplatin-induced nephrotoxicity in rats. Drug Des Devel Ther. 11:901-908.
7. Abo-Elmaaty AMA, Behairy A, El-Naseery NI, Abdel-Daim MM. (2020) The protective efficacy of vitamin E and cod liver oil against cisplatin-induced acute kidney injury in rats. Environ Sci Pollut Res Int. 27(35):44412-44426.
8. Aboubakr M, Abdelkader A, Habotta OA, Adel N, Emam MA, Abdelhiee EY, Shanab O, Shoghy K, Elnoury H, Soliman MM, Ibrahim SF, Abdeen A. (2021) Cefepime and diclofenac sodium combined treatment-potentiated multiple organ injury: Role of oxidative damage and disrupted lipid metabolism. J Biochem Mol Toxicol. 35(12): e22929. doi: 10.1002/jbt.22929.
9. Aboubakr M, Elsayd F, Soliman A, Fadl SE, El-Shafey A, Abdelhiee EY. (2020) L-Carnitine and vitamin E ameliorate cardiotoxicity induced by tilmicosin in rats. Environ Sci Pollut Res Int. 27(18):23026-23034.
10. Aboubakr M, Ibrahim SS, Said AM, Elgendey F, Anis A (2019) Neuroprotective effects of clove oil in acrylamide induced neurotoxicity in rats. Pak Vet J. 39 (1):111-115.
11. Abugomaa A, Elbadawy M. (2022) Olive leaf extract modulates glycerol-induced kidney and liver damage in rats. Environ Sci Pollut Res Int. 27(17):22100-22111.
12. Acaroz U, Ince S, Arslan-Acaroz D, Gurler Z, Kucukkurt I, Demirel HH, Arslan HO, Varol N, Zhu K. (2018) The ameliorative effects of boron against acrylamide-induced oxidative stress, inflammatory response, and metabolic changes in rats. Food Chem Toxicol. 118:745-752.
13. Bancroft JD, Gamble M. (2008) Theory and Practice of Histological Techniques. 6th ed. China: Churchill Livingstone, Elsevier.
14. Bin-Jumah MN, Al-Huqail AA, Abdelnaeim N, Kamel M, Fouda MMA, Abulmeaty MMA, Saadeldin IM, Abdel-Daim MM. (2021) Potential protective effects of *Spirulina platensis* on liver, kidney, and brain acrylamide toxicity in rats. Environ Sci Pollut Res Int. 28(21):26653-26663.
15. Böhm V. (2018) Vitamin E. *Antioxidants*. 7(3):44. https://doi.org/10.3390/antiox7030044
16. Chin KY, Ima-Nirwana S. (2019) The Role of Tocotrienol in Preventing Male Osteoporosis-A Review of Current Evidence. Int J Mol Sci. 20(6):1355. doi:10.3390/ijms20061355
17. El Fakahany GA, Nassar SA, Elballat SE. (2021) Alterations in kidney of albino rat due to acrylamide exposure and the possible protective role of L-arginine (biochemical, histological, immunohistochemical and molecular study). Sys Rev Pharm 12(03):744-752
18. Elblehi SS, El Euony OI, El-Sayed YS. (2020) Apoptosis and astrogliosis perturbations and expression of regulatory inflammatory factors and neurotransmitters in acrylamide-induced neurotoxicity

under ω3 fatty acids protection in rats. Neurotoxicology, 76:44-57
19. Elhelaly AE, AlBasher G, Alfarraj S, Almeer R, Bahbah EI, Fouda MMA, Bungău SG, Aleya L, Abdel-Daim MM. (2019) Protective effects of hesperidin and diosmin against acrylamide-induced liver, kidney, and brain oxidative damage in rats. Environ Sci Pollut Res Int. 26(34):35151-35162.
20. Elkomy A, Aboubakr M, Ibrahim S, Abdelhamid Y (2018) Protective effects of *Syzygium aromaticum* oil (Clove) against acrylamide induced hepatic, renal, and testicular toxicity in rats. Int. J. Pharmacol. Toxicol. 6:12-17.
21. Elkomy A, Aboubakr, Yara Medhat Y, Abugomaa A, Elbadawy M (2019) Nephroprotective Effects of Cinnamon and/or Parsley Oils against Gentamicin-Induced Nephrotoxicity in Rats. J Anim Vet Adv. 18: 201-207.
22. Elsayed A, Elkomy A, Alkafafy M, Elkammar R, El-Shafey A, Soliman A, Aboubakr M. (2022a) Testicular toxicity of cisplatin in rats: ameliorative effect of lycopene and N-acetylcysteine. Environ Sci Pollut Res Int. 29(16):24077-24084.
23. Elsayed A, Elkomy A, Alkafafy M, Elkammar R, Fadl SE, Abdelhiee EY, Abdeen A, Shaheen H, Soliman A and Aboubakr M (2022b) Ameliorating effect of lycopene and N-acetylcysteine against cisplatin-induced cardiac injury in rats. Pak Vet J,42(1):107-111.
24. Elsayed A, Elkomy A, Elkammar R, Youssef G, Abdelhiee EY, Abdo W, Fadl SE, Soliman A, Aboubakr M. (2021) Synergistic protective effects of lycopene and N-acetylcysteine against cisplatin-induced hepatorenal toxicity in rats. Sci Rep. 11(1):13979. doi: 10.1038/s41598-021-93196-7.
25. Elsayed MG, Elkomy AA, Gaballah MS, Elbadawy M. (2014) Nephrotoxicity of cefepime: A new cephalosporin antibiotic in rats. J Pharmacol Pharmacother. 5(1):33-38.
26. Elyasi S, Khalili H, Hatamkhani S, Dashti-Khavidaki S. (2013) Prevention of vancomycin induced nephrotoxicity: a review of preclinical data. Eur J Clin Pharmacol. 69(4):747-754.
27. Erdemli ME, Gul M, Altinoz E, Zayman E, Aksungur Z, Bag HG. (2017) The protective role of crocin in tartrazine induced nephrotoxicity in Wistar rats. Biomed Pharmacother. 96:930-935.
28. Fan H, Le JW, Zhu JH. (2020) Protective Effect of N-Acetylcysteine Pretreatment on Acute Kidney Injury in Septic Rats. J Surg Res. 254:125-134.
29. Ghlissi Z, Hakim A, Mnif H, Zeghal K, Rebai T, Boudawara T, Sahnoun Z. (2018) Combined use of Vitamins E and C improve nephrotoxicity induced by colistin in rats. Saudi J Kidney Dis Transpl. 29(3):545-553.
30. Ghlissi Z, Hakim A, Sila A, Mnif H, Zeghal K, Rebai T, Bougatef A, Sahnoun Z. (2014) Evaluation of efficacy of natural astaxanthin and vitamin E in prevention of colistin-induced nephrotoxicity in the rat model. Environ Toxicol Pharmacol. 37(3):960-966.
31. Ghorbel I, Elwej A, Fendri N, Mnif H, Jamoussi K, Boudawara T, Grati Kamoun N, Zeghal N. (2017) Olive oil abrogates acrylamide induced nephrotoxicity by modulating biochemical and histological changes in rats. Ren Fail. 39(1):236-245.
32. Mansour SA, Mohamed DA, Gamet-Payrastre L. (2014) Effects of indirect exposure of mice pups to endosulfan via their dams during gestation and lactation periods and the ameliorative effect of vitamin E. Hum Exp Toxicol. 33(9):911-927.
33. Pedrycz A, Boratynski Z and Krasowski A (2010) Immunohistochemical assessment of the effects of larginine as a nitric oxide (NO) substrate on caspase 3 expression in rats' renal tubular cells. Bulletin of the Veterinary Institute in Puławy. 54(3): 441- 443.
34. Rajeh NA, Al-Dhaheri NM. (2017) Antioxidant effect of vitamin E and 5-aminosalicylic acid on acrylamide induced kidney injury in rats. Saudi Med J. 38(2):132-137.
35. Rivadeneyra-Domínguez E, Becerra-Contreras Y, Vázquez-Luna A, Díaz-Sobac R, Rodríguez-Landa JF. (2018) Alterations of blood chemistry, hepatic and renal function, and blood cytometry in acrylamide-treated rats. Toxicol Rep. 5:1124-1128.
36. Sengul E, Gelen V, Yildirim S, Tekin S, Dag Y. (2021) The Effects of Selenium in Acrylamide-Induced Nephrotoxicity in Rats: Roles of Oxidative Stress, Inflammation, Apoptosis, and DNA Damage. Biol Trace Elem Res. 199(1):173-184.
37. Siddarth M, Chawla D, Raizada A, Wadhwa N, Banerjee BD, Sikka M. (2018) Lead-induced DNA damage and cell apoptosis in human renal proximal tubular epithelial cell: Attenuation via N-acetyl cysteine and tannic acid. J Biochem Mol Toxicol. 32(3):e22038. doi: 10.1002/jbt.22038.
38. Soliman MM, Aldhahrani A, Gaber A, Alsanie WF, Mohamed WA, Metwally MMM, Elbadawy M, Shukry M. (2022a) Ameliorative impacts of chrysin against gibberellic acid-induced liver and kidney damage through the regulation of antioxidants, oxidative stress, inflammatory cytokines, and apoptosis biomarkers. Toxicol Res (Camb). 11(1):235-244.
39. Soliman MM, Gaber A, Alsanie WF, Mohamed WA, Metwally MMM, Abdelhadi AA, Elbadawy M, Shukry M. (2022b) Gibberellic acid-induced hepatorenal dysfunction and oxidative stress: Mitigation by quercetin through modulation of antioxidant, anti-inflammatory, and antiapoptotic activities. J Food Biochem. 46(2):e14069. doi: 10.1111/jfbc.14069.
40. Song J, Zhao M, Liu X, Zhu Y, Hu X, Chen F. (2013) Protection of cyanidin-3-glucoside against oxidative stress induced by acrylamide in human MDA-MB-231 cells. Food Chem Toxicol. 58:306-310.
41. Tenório MCDS, Graciliano NG, Moura FA, Oliveira ACM, Goulart MOF. (2021) *N*-Acetylcysteine (NAC): Impacts on Human Health. Antioxidants. 10(6):967. doi: 10.3390/antiox10060967.
42. Uthra C, Shrivastava S, Jaswal A, Sinha N, Reshi MS, Shukla S. (2017) Therapeutic potential of quercetin against acrylamide induced toxicity in rats. Biomed Pharmacother. 86:705-714.
43. Yalçın A, Gürel A. (2021) Effects of N-acetylcysteine on kidney tissue, matrix metalloproteinase-2, irisin and oxidative stress in a diabetes mellitus model. Biotech Histochem. 96(8):616-622.
44. Yue Z., Chen Y., Song Y., Zhang J., Yang X., Wang J., Li L., Sun Z. (2020) Effect of acrylamide on glucose homeostasis in female rats and its mechanisms. Food Chem. Toxicol. 135:110894. doi: 10.1016/j.fct.2019. 110894.