Continental Veterinary Journal
Journal homepage: www.cvetj.com

Research Article

Glyphosate induced renal toxicity and its amelioration with vitamin C in rats

M. Lakshmi Namratha[1], M. Lakshman[2*], M. Jeevanalatha[3], B. Anil Kumar[4] and Saba Rashid[5]

[1]Department of Veterinary Pathology, P.V. Narsimha Rao Telangana Veterinary University, India
[2]Department of Veterinary Pathology, P.V. Narsimha Rao Telangana Veterinary University, India
[3]Department of Veterinary Pathology, P.V. Narsimha Rao Telangana Veterinary University, India
[4]Department of Veterinary Pharmacology and Toxicology, P.V. Narsimha Rao Telangana Veterinary University, India
[5]Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan

*Correspondence: mekala_bry@yahoo.com

ARTICLE INFO

ARTICLE HISTORY: CVJ-22-0405

Received 31 August 2022
Revised 08 December 2022
Accepted 08 December 2022
Published online 12 December 2022

Keywords:

Glyphosphate
Creatinine
Malondialdehyde
Free radicals
Wistar rats

ABSTRACT

The main clinical effects of consuming glyphosate are hepatotoxicity and renal toxicity. Vitamin C, a well-known antioxidant, helps to prevent cellular damage brought on by free radicals. The aim of this study was to see how glyphosate affected renal tissues in male albino Wistar rats at a dosage of 500 mg/kg body weight (1/10 LD50) and how vitamin C responded at a dose of 250 mg/kg body weight. During these three weeks, the experimental organisms received daily oral treatments. The histology and ultrastructure pathologies of renal tissues, tissue oxidative stress parameters, and serum biochemical assays were investigated. A considerable rise in the levels of the blood enzymes urea and creatinine in the treated group was observed during this study. Malondialdehyde concentration, reduced glutathione, and superoxide dismutase levels were measured to assess oxidative stress in renal tissues. Kidney segment histopathology and ultrastructure pathology showed significant alterations. However, vitamin C treatment had a slight to moderately ameliorative effect on the examined parameters.

To Cite This Article: Namratha ML, M Lakshman, M Jeevanalatha, BA Kumar and S Rashid, 2022. Glyphosate induced renal toxicity and its amelioration with vitamin C in rats. Continental Vet J, 2(2):81-89.

Introduction

Herbicides have been an integral part of the expanding agrochemical pesticide industry over the past 20 years. Water-soluble, non-selective herbicide glyphosate [N-(phosphomonomethyl) glycine] (GLP) is applied to foliage and causes the weeds to die (Kremer and Means 2009). One of the most widely used and environmentally harmful herbicides is glyphosate, which is sold under the brand name Roundup®. 15% polyoxyethylene amine (POEA) and other unidentified surfactants are combined in Roundup® (Howe et al. 2004). According to studies, this mixture is more harmful than glyphosate by itself (Williams et al. 2000; Howe et al. 2004; Santos et al. 2005). Because of its widespread use, glyphosate is now more prevalent in the environment, surface water, and groundwater (Benbrook 2016). Cytochrome P450 and two additional enzymes (G-6-P dehydrogenases and glutathione-S-transferases), which are critical to the body's detoxification of toxins, were negatively impacted by Roundup (Acquavella et al. 2004). GLP disrupts the body's normal DNA repair machinery and damages DNA in human cells as an endocrine disruptor, which leads to genomic instability and the spread of cancer. The main cause of GLP's toxicity is the decoupling of oxidative phosphorylation (Ikpeme et al. 2012).

By generating reactive oxygen species (ROS) such as hydroxyl radicals (OH), superoxide anions, and hydrogen peroxide, a variety of pollutants can disrupt biological systems (H_2O_2) (Ahmad et al. 2000; Harish and Murugan 2011). Animals have an antioxidant defence system that consists of enzymes such as, catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx), as well as non-enzymatic antioxidants such as non-protein thiols, notably glutathione (GSH). One of most harmful effects of oxidative stress is membrane lipid peroxidation, which happens when the balance between pro-oxidants and antioxidants shifts (LPO) (Scandalios 2005).

The present study's goal is to investigate the negative consequences of glyphosate on the male Wistar albino

rats' renal tissues by evaluating the serum biochemistry tests, oxidative stress indices, histopathology, and ultrastructure pathology due to the dearth of literature on glyphosate toxicity. A powerful antioxidant known as vitamin C was employed to mitigate the damage caused by free radicals brought on by glyphosate.

Materials and Methods

Chemical compounds

A commercial glyphosate formulation named Roundup® was acquired from Hyderabad-30's Professor Jayashankar Telangana State Agriculture University (PJTSAU) Seed Research and Technology Centre (SRTC), while vitamin C was obtained from Mumbai, India's S.D. Fine-Chem Ltd. Glyphosate is the primary ingredient in Roundup® at a 41 percent concentration.

Experimental animals

Wistar albino adult male rats, raised at Jeeva Life Sciences (an ISO 9001:2015-certified company), Hyderabad, weighed 200–240 g for the study. Animals underwent one week of acclimatization. A 12-hour cycle of light and dark was employed in the lighting cycle, and the temperature was kept at 22°C. Throughout the trial, all of the rats received a regular pellet meal and unlimited access to deionized water.

Animal ethics

All of the animals were treated humanely in line with the requirements specified by the Institutional Animal Ethics Committee (IAEC). The study was conducted after receiving IAEC's previous clearance (01/2019).

Treatment

We randomly divided 48 adult male rats into four groups (G1-G4), each having twelve (12) individuals. This was preferred since the normal sample size for scientific experiments with inbred rodents is between 5 and 7 (Kubota and Wakana 2011). Group 1 was regarded as control and was given distilled water, Group 2 was given GLP (500 mg/kg body weight), Group 3 was given vitamin C (250 mg/kg body weight) and Group 4 was given GLP + vitamin C (500 mg/kg body weight + 250 mg/kg body weight), daily orally for three weeks. After one and three weeks of therapy, six rats from each group were sacrificed by cervical dislocation. Quickly separated, weighed, and placed in a cold bath were the kidneys.

Biochemical evaluation

Without the application of anesthesia, 1-1.5 mL of blood samples were taken from the rats using ocular puncture at the conclusion of the first and third weeks. Animal blood samples were drawn into simple tubes, allowed to clot, and then centrifuged to extract the serum. The serum was then stored at 4°C until it was needed. Blood urea nitrogen (BUN) and creatinine were two biochemical parameters that were examined using Erba Mannheim Diagnostic kits and an automated serum analyzer (Star 21 Plus, Rapid Diagnostic Pvt. Ltd.).

Oxidative stress indices

To get 10% homogenate for all the tissue antioxidant profiles, one gramme of kidney tissue was placed into a tissue homogenizer along with 10 mL of 0.2 M Tris HCl buffer (pH 7.2). Malondialdehyde and other lipid peroxidation end products were used to measure the tissue oxidation (Balasubramanian et al. 1988). Using procedures, tissue protein was estimated (Lowry et al. 1951), kidney SOD activity was assayed by the method of Madesh and Balasubramanian (1998) and reduced glutathione (GSH) (Moron et al. 1979) to know the antioxidant status.

Histopathological studies

Following euthanasia, samples of kidney tissue were removed surgically from the animals. All samples were fixed in Neutral Buffered Formalin (NBF) at a concentration of 10%, then washed, dehydrated in alcohol, clarified in xylene, and mounted in paraffin blocks. Following the normal approach (Luna 1968), the tissues were cut into 5µm slices, stained with hematoxylin and eosin (H&E), mounted in neutral Dibutyl phthalate Xylene (DPX) media, and examined under a light microscope.

Ultrastructure Pathology

As soon as the kidneys were sacrificed, they were cut into thin slices, fixed in 2.5 percent glutaraldehyde (PBS-based), and prepared for TEM and SEM analysis according to procedure (Lakshman 2017; Lakshman 2019).

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 16.0 was used to do a one-way analysis of variance (ANOVA) on the data gathered from the experimental animals. The significance level was set at P 0.05, and the results were presented as mean + SE (Snedecor and Cochran 1994).

Results

When compared to the control group, the BUN and serum creatinine levels in the rats exposed to GLP for one and three weeks were significantly increased. BUN and creatinine levels were moderately reduced by co-administration of vitamin C (G4) (Table 1).

When compared to the control rats, the MDA levels in the kidney homogenates of the rats given GLP for one and three weeks were significantly ($P<0.05$) higher. Following treatment, there was a discernible decrease in vitamin C (Table 2).

When compared to the control group, there was a significant ($P<0.05$) decrease in the mean values of SOD and GSH in renal homogenates of rats treated with glyphosate for one and three weeks. Only half of the enzyme activity was restored when vitamin C was added. The enzymes' activities were partially recovered when vitamin C was also provided. The rats given vitamin C alone (G3) displayed results comparable to the control group (Table 2).

The kidneys of rats given glyphosate treatment for a week revealed hyaline casts, enlarged Bowman's gap, glomerular atrophy, and damaged tubules. After three weeks of treatment, there was congestion, fibrous tissue proliferation, glomerulus engorgement, intratubular hemorrhages, significant round cell infiltration, and tubular necrosis with pyknotic

nuclei. In kidney sections of rats treated with glyphosate and vitamin C, mild tubular regeneration was seen coupled with lesser intensity lesions (Fig 1). Anisocytosis and poiklocytosis of mitochondria with loss of cristae were seen in kidney sections from rats given glyphosate treatment for a week, as well as epithelial cells with pyknotic and enlarged nuclei. After three weeks of treatment, a thin portion of the kidney revealed deformed tubules, enlarged microcapillary walls, and increased basement membrane tubule thickness. Co-administration of vitamin C resulted in modest tubule and cytosol reconstruction, a large number of vesicular mitochondria, the loss of epithelial junctions, and increased tubular membrane thickness (Fig. 2).

One week after glyphosate treatment, SEM of kidney sections revealed engorged glomeruli and tubular necrosis. After three weeks of treatment, mononuclear cell infiltration, tubular necrosis, and fibrous tissue proliferation were evident (Fig. 3).

Table 1: Serum biochemical parameters in different groups at different time intervals

GROUP	BUN (mg/dL)		CREATININE (mg/dL)	
	Day-7	Day-21	Day-7	Day-21
Group 1	37.83 ± 1.30^{a}	38.67 ± 0.91^{a}	0.56 ± 0.05^{a}	0.8 ± 0.03^{a}
Group 2	56.67 ± 1.49^{b}	64.83 ± 2.08^{c}	1.21 ± 0.08^{c}	1.75 ± 0.08^{c}
Group 3	38.33 ± 1.11^{a}	40.5 ± 0.92^{a}	0.73 ± 0.06^{a}	0.83 ± 0.03^{a}
Group 4	53.17 ± 0.79^{b}	56.17 ± 3.12^{b}	0.98 ± 0.06^{b}	1.03 ± 0.07^{b}

Values are Mean ± SE (n=6); One-way ANOVA

Means with different superscripts in a column differ significantly at P<0.05

Table 2: Oxidative stress indices in different groups at different time intervals

PARAMETER	GROUP-1		GROUP-2		GROUP-3		GROUP-4	
	DAY-7	DAY-21	DAY-7	DAY-21	DAY-7	DAY-21	DAY-7	DAY-21
MDA(µM/ mg of protein)	3.70 ± 0.31^{a}	3.80 ± 0.39^{b}	7.35 ± 0.18^{c}	8.30 ± 0.18^{d}	3.43 ± 0.77^{a}	3.35 ± 0.76^{a}	6.46 ± 0.11^{b}	7.35 ± 0.14^{c}
GSH (µM/mg protein)	10.35 ± 0.08^{c}	10.57 ± 0.05^{c}	8.45 ± 0.11^{a}	7.35 ± 0.06^{a}	10.49 ± 0.04^{c}	10.61 ± 0.07^{c}	8.85 ± 0.03^{b}	8.63 ± 0.14^{b}
SOD (U/mg protein)	11.45 ± 0.13^{c}	11.59 ± 0.13^{c}	9.68 ± 0.14^{a}	9.68 ± 0.14^{a}	11.66 ± 0.14^{c}	11.82 ± 0.12^{c}	10.53± 0.19^{b}	9.35 ± 0.27^{b}

Values are Mean ± SE (n=6); One way ANOVA

Means with different superscripts in a column differ significantly at P<0.05

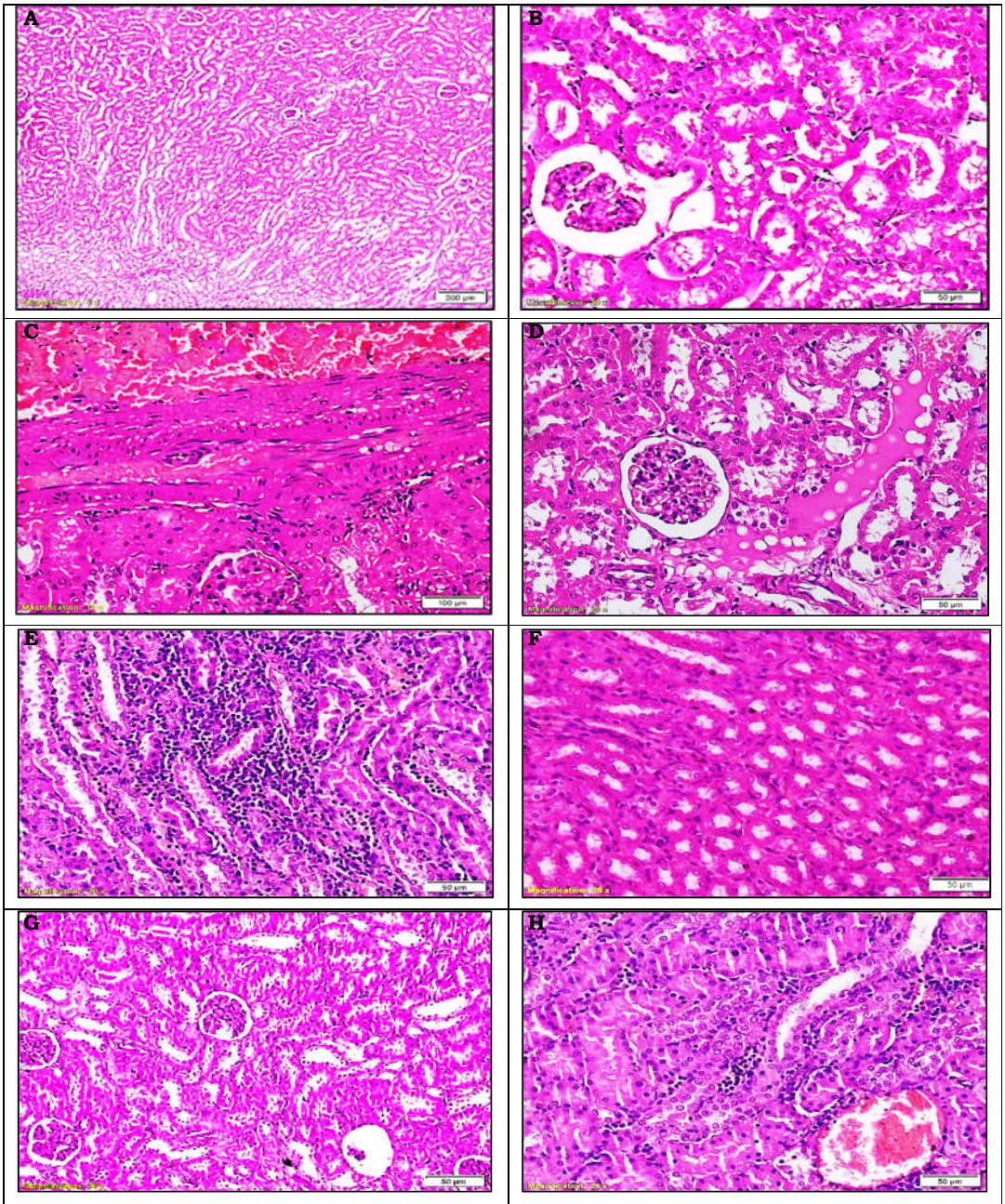

Fig. 1: Histological analysis of kidneys of different groups (H&E).
A: Group 1: Kidney showing normal glomeruli and tubules (x40)
B: Group 2, Day 7: Kidney section showing glomerular atrophy, increased Bowman's space, degenerated tubules and hyaline casts (x200)
C: Group 2, Day 21: Kidney section showing congestion, fibrous tissue proliferation, engorgement of glomerulus and tubular necrosis (x100)
D: Group 2, Day 21: Kidney section showing edema, vacuolar degeneration in glomerulus, sloughing of tubular epithelium and mild fibrous tissue proliferation (x200)
E: Group 2, Day 21: Kidney section showing intertubular hemorrhages, marked round cell infiltration and tubular necrosis with pyknotic nuclei (x200)
F: Group 3, Day 21: Kidney section showing normal appearance of the tubules (x200)
G: Group 4, Day 7: Kidney section showing mild regeneration of tubules and glomerular atrophy (x200)
H: Group 4, Day 21: Kidney section showing congestion, mild round cell infiltration and tubular necrosis with pyknotic nuclei (x200)

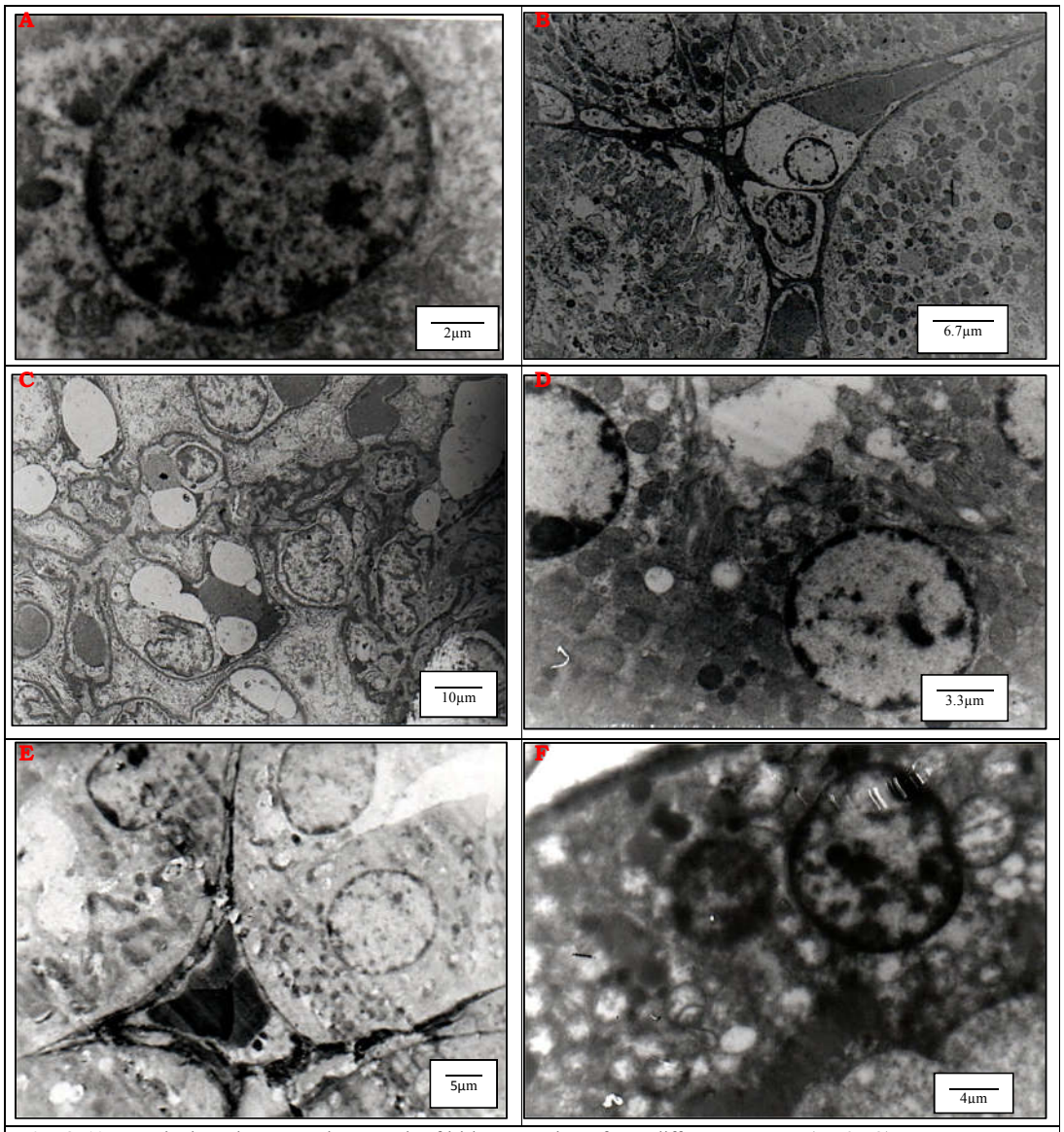

Fig. 2: Transmission electron micrograph of kidney sections from different groups (UA&LC)
A: Group 1, Day 21: A thin slice of the kidney displaying mitochondria in a normal state (9650x)
B: Group 2, Day 7: A thin section of the kidney reveals dilated intratubular space with blood cells, thickened capillary walls, enlarged and dilated RER cisternae, anisocytosis and poiklocytosis of mitochondria with lack of cristae, and epithelial cells with pyknotic and swollen nuclei (2895x)
C: Group 2, Day 21: Renal ultrathin slice demonstrating deformed tubules, thickening microcapillary wall, and increased thickness of tubule basement membrane (1930x)
D: Group 3, Day 21: a thin segment of kidney epithelial cells displaying a normal nucleus and uniformly sized mitochondria (5790x)
E: Group 4, Day 7: Kidney ultrathin section demonstrating dilated intertubular junctions, tubular epithelial cell necrosis, and kidney deterioration (3860x)
F: Group 4, Day 21: Kidney ultrathin section demonstrating epithelial junction loss, modest reconstruction of tubules, cytosol, pyknotic nuclei, many vesicular mitochondria, and thickening of the tubular membrane (4825x)

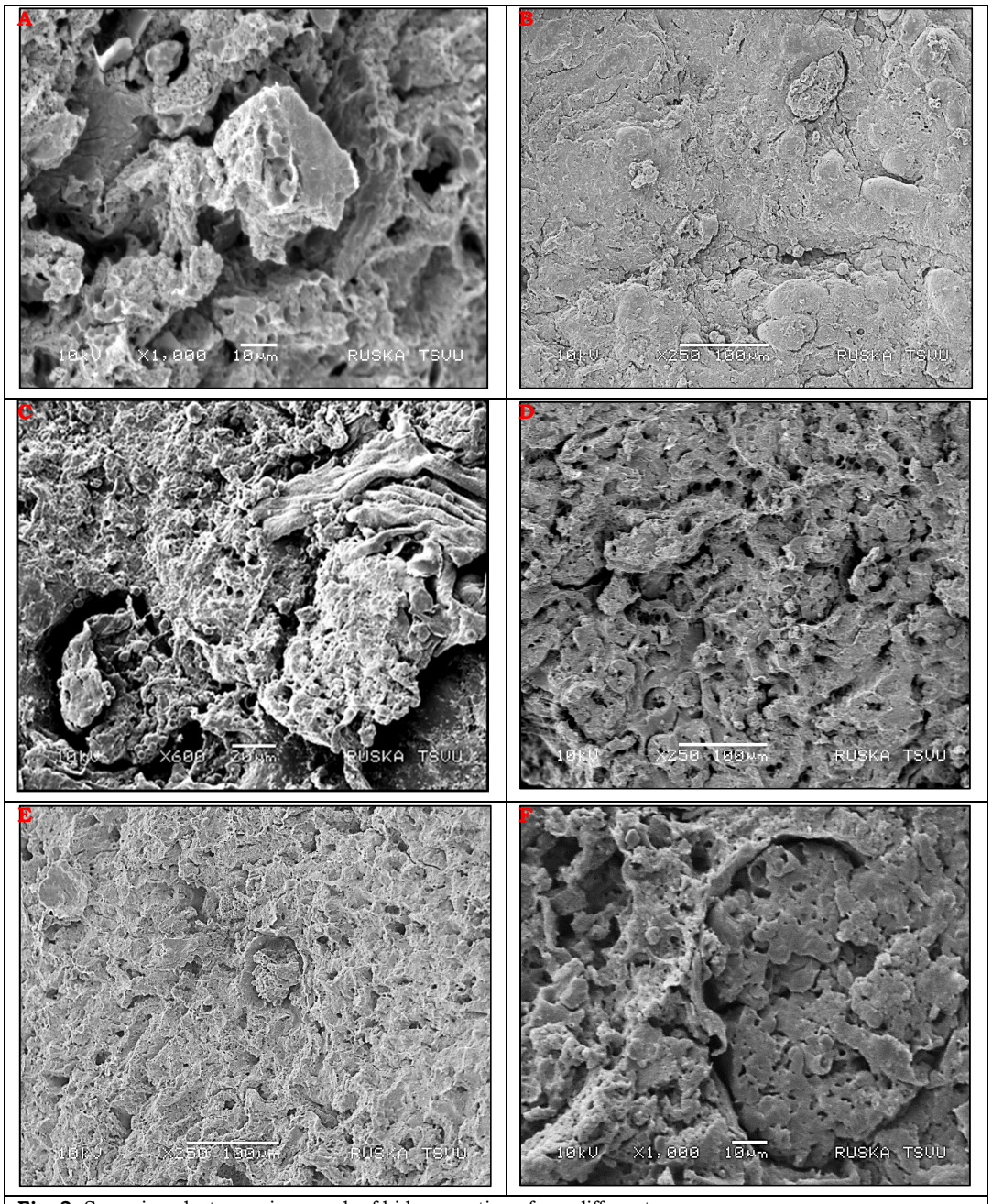

Fig. 3: Scanning electron micrograph of kidney sections from different groups
A: Group 1, Day 21: Ultrathin section of kidney showing normal glomerulus and intact Bowman's capsule
B: Group 2, Day 7: Ultrathin section of kidney showing engorged glomerulus and tubular necrosis
C: Group 2, Day 21: Ultrathin section of kidney showing infiltration of MNCs, tubular necrosis and fibrous tissue proliferation
D: Group 3, Day 21: Ultrathin section of kidney showing normal intact tubules
E: Group 4, Day 7: Ultrathin section of kidney showing atrophy of glomerulus and narrow tubules
F: Group 4, Day 21: Ultrathin section of kidney showing glomerular engorgement with dilated tufts

Discussion

Notably, farmers use pesticides frequently to eradicate weeds all around the world. During application and container disposal, both humans and animals are directly and indirectly exposed, which causes mortality. The results of the current investigation showed that kidney function in rats exposed to glyphosate was severely impaired, as evidenced by the obvious histological and ultrastructural changes. Anorexia, decreased water intake, lethargy, moderate watery diarrhea, and weakness were some of the clinical symptoms in rats exposed to glyphosate displayed. However, no fatalities were recorded during the course of the investigation.

To monitor nephrotoxicity, serum creatinine and BUN levels are employed as an early and sensitive

biomarker (Lall et al. 1997). Cavusoglu et al. (2011) argued that glomerular filtration impairment and renal tubular injury are to blame for the rise in BUN and serum creatinine levels. El-Shenawy (2009) stated that when tissues are disrupted, the enzyme xanthine dehydrogenase is transformed to xanthine oxidase by the oxidation of crucial "SH" groups, causing kidney damage owing to a huge increase in free radical generation and ATP depletion as a result of tissue hypoxia. Weir et al. (2003) reported that the conversion of hypoxanthine to uric acid, xanthine, and superoxide is catalyzed by xanthine oxidase. This might be one of the causes of the increased uric acid levels in the rats treated with glyphosate and roundup. Considered to be a powerful antioxidant molecule is uric acid (Nieto et al. 2000). The increased serum uric acid levels in glyphosate-treated animals may be a result of a protective mechanism to offset the increased oxidative stress. According to the results of the current study, rats given glyphosate treatment exhibited significantly higher levels of BUN and serum creatinine. These findings can be linked to histopathological findings, specifically tubular degeneration, necrosis, along with fibrosis and glomerulus atrophy, which may explain the rise in serum enzyme levels. Supposedly, hypoxia and excessive ROS generation are also to blame for this. Cavusoglu et al. (2011), Jasper et al. (2012) and Youness et al. (2016) also published similar results. The ameliorative group's lower BUN and serum creatinine levels are a sign of improved cell respiration and vitamin C's antioxidant properties.

Oxidative stress is the term for the *in vivo* imbalance of oxidation and anti-oxidation (Hou et al. 2013). Oxidative damage can occur when defensive mechanisms are insufficient to neutralize ROS; membrane LPO is one of the more severe forms (Ahmad et al. 2004). The MDA is a biomarker for LPO and the stable metabolite of LPO products (Sun et al. 2001). The results of the current study showed a significant rise in the levels of MDA in the kidney tissues of glyphosate-treated rats. This rise in MDA levels may be attributable to increased LPO, which in turn causes an increase in the intracellular accumulation of ROS. MDA, a byproduct of lipid peroxidation, is known to be produced by ROS acting on the unsaturated fatty acids of the phospholipids that make up membrane components. The components of the herbicide directly interact with the cytoplasmic membrane of the kidney cells, causing a disruption in the structure of the membrane, which is the mechanism underlying this peroxidation (Dedeke et al. 2018). Vitamin C (G4) co-administration in the current study significantly reduced MDA levels, demonstrating its antioxidant properties. Vitamins C and E and N-acetyl-L-cysteine (NAC) are antioxidants that may lessen the harmful effects of GLP (Youness et al. 2016). In addition to acting as an antioxidant, vitamin C also acts as a pro-oxidant. Vitamin C securely interacts with free radicals and breaks down chain reactions before damaging essential components. Additionally, it prevents other oxidative processes and removes free radical intermediates (Ikpeme et al. 2012).

Additionally, a significant amount of the renal disease is linked to the drop in intracellular GSH content (Atef 2011). Therefore, GSH content is crucial for cell survival. In addition, glutathione peroxidase uses it as a substrate. Tripeptide glutathione is one of the most significant modulatory mechanisms for neutralizing free radicals and preventing the attack of electrophilic xenobiotics on cellular macromolecules (Cnubben et al. 2001). In the current investigation, there was a noticeable decrease in GSH levels after glyphosate treatment is in accordance with the observations of El-Shenawy (2009), Cavusoglu et al. (2011) and Tang et al. (2017). It's possible to suppose that Roundup increased cell death by increasing oxidative processes and creating an oxidative imbalance by lowering GSH levels (El-Shenawy 2009). Due to its capacity to cause mitochondrial damage and oxidative stress in kidney homogenates, the present study's considerable drop in the mean values of GSH and SOD can be explained. The theory is supported by the ultrastructural alterations in the mitochondria of tubular epithelial cells in the current investigation. When compared to group 2, the rats in group 4 had a little to moderate improvement, demonstrating the preventive effect of vitamin C against GLP-induced nephrotoxicity.

Namratha et al. (2019) revealed that kidney weights in rats given glyphosate (500 mg/kg body weight, orally) orally for three weeks showed a substantial drop, followed by marked congestion and an atrophied look. The multiple histological impairments to the kidney tissues could have been caused by the increased LPO, decreased antioxidant defenses, increased blood urea, and creatinine, which were all seen in rats treated to the Roundup formulation in this investigation. Rats exposed to Roundup showed kidney sections with distorted renal-cortical histoarchitecture, engorged glomeruli, intratubular fibrous tissue proliferation, noticeable round cell infiltration, glomerular atrophy with vacuolar degeneration, and severity that was directly inversely proportional to the number of days of exposure. Cavusoglu et al. (2011); Karimi et al. (2014); Tizhe et al. (2014) and Tang et al. (2017) also reported similar findings. Wunnapuk et al. (2014) confirmed that during the acute stages of Roundup poisoning, renal cell death mechanisms occurred in tubules and glomeruli. Therefore, taking the herbicide Roundup by mouth has the potential to not only harm the kidney but also to result in chronic renal disease and eventual kidney death. The modifications in the current study may have resulted from excessive ROS production, which outweighed endogenous antioxidants produced by the body and had stronger effects on calcium influx and the Na+/K+ ion transport system. The ATP depletion seen as a result of the cytotoxic effects of GLP, presumably through increased ROS or oxidative stress, may have been the cause of the tubular necrosis. When compared to group 2, these glyphosate-induced nephrotoxic effects were least pronounced in group 4. Despite vitamin C's antioxidant qualities, these lesions may be a result of the GLP's propensity for toxicity. These histological changes are related to the ultrastructure observations. According to Langeswaran et al. (2012), in addition to controlling membrane permeability and ion transport across the cellular membrane at the expense of ATP through hydrolysis, ATPases are crucial enzymes that fuel metabolic energy for life processes. These ion-dependent ATPases are inhibited, which causes abnormalities in ion

homeostasis that disrupt signal transduction, affect cellular metabolism, alter cell membrane integrity and permeability, increase membrane fluidity, and impede essential processes (Chodon et al. 2008). The herbicide Roundup has the ability to decrease the activities of membrane-bound enzymes (Ca-ATPase, Mg-ATPase, total ATPase, and Na/K-ATPase) in the kidney of the exposed animal, suggesting disruptions in the cellular metabolism of the kidney and changes in the kidney cell membrane and integrity brought on by the herbicide compositions (Dedeke et al. 2018). Overproduction of ROS, which may have caused mitochondrial malfunction, can be blamed for the ultrastructural alterations observed in glyphosate-treated rats. We can draw the conclusion that vitamin C alone was unable to entirely counteract the damaging effects of GLP caused by ROS and other molecular mechanisms, such as apoptosis.

Conclusion

The findings of the current study demonstrated that exposure to a commercial formulation of the herbicide Roundup containing glyphosate at different time points led to severe renal damage brought on by increased oxidative stress, which increased serum urea and creatinine as well as histopathological and ultrastructural alterations. It's not yet apparent if glyphosate's presence in the Roundup formulation has a synergistic effect or whether another chemical in the formulation is primarily to blame for the documented nephrotoxicity. Despite vitamin C's antioxidant abilities, co-administration of the vitamin (250 mg/kg body weight) showed a minor improvement in biochemical and oxidative stress markers as well as a modest improvement in histological and ultrastructural alterations, indicating the possible toxicity of GLP. Although vitamin C has potent antioxidant properties, supplementation by itself has not completely corrected the negative effects caused by GLP. Research is still needed to determine the most effective ameliorative agent or combination of agents that can reduce the negative effects of GLP.

Acknowledgment

The facilities required for a successful clinical research project were provided by PV Narsimharao Telangana Veterinary University, and the authors genuinely appreciate and thank them for their assistance.

Funding: No

Competing Interest: The authors have no relevant financial or non financial interests to disclose.

Author Contribution: All authors contributed to the study conception, design and analysis.

References

Acquavella JF, Bruce H, Alexander BH, Mandel JS, Gustin C and Baker B, 2004. Glyphosate biomonitoring for farmers and their families: results from the farm family exposure study. Environmental Health Perspectives 112: 321–326.

Ahmad I, Hamid T, Fatima M, Chand HS, Jain SK, Athar M and Raisudin S 2000. Induction of hepatic antioxidants in freshwater catfi sh (Channa punctatus Bloch) is a biomarker of paper mill effluent exposure. Biochimica et Biophysica Acta 1523: 37–48.

Ahmad I, Pacheco M and Santos MA, 2004. Enzymatic and nonenzymatic antioxidants as an adaptation to phagocyte induced damage in Anguilla anguilla L. following in situ harbor water exposure. Ecotoxicology and Environmental Safety 57 (3): 290-302.

Atef MA, 2010. Physiological and histopathological investigations on the effects of α-lipoic acid in rats exposed to Malathion. Journal of Biomedicine and Biotechnology 203503: 1–8.

Balasubramanian KA, Manohar M and Mathan VI, 1988. An unidentified inhibitor of lipid peroxidation in intestinal mucosa. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism 962: 51-58.

Benbrook CM, 2016. Trends in glyphosate herbicide use in the United States and globally. Environmental Science Europe 28: 3

Cavusoglu K, Yapar K, Oruc E and Yalcin E, 2011. Protective effect of Ginkgo biloba L. leaf extract against glyphosate toxicity in Swiss albino mice. Journal of Medicinal Food 14 (10): 1263-1272.

Chodon D, Arumugam A, Rajasekaran D and Dhanapal S, 2008. Effect of genistein on modulating lipid peroxidation and membrane-bound enzymes in N-nitrosodiethylamine-induced and phenobarbital-promoted rat liver carcinogenesis. Journal of Health Science 54 (2):137-42.

Cnubben NH, Rietjens IM, Wortelboer H, van Zanden, J and van Bladeren, PJ, 2001. The interplay of glutathione-related processes in antioxidant defense. Environmental Toxicology and Pharmacology 10 (4): 141-152.

Dedeke GA, Owagboriaye FO, Ademolu KO, Olujimi OO and Aladesida AA, 2018. Comparative assessment on mechanism underlying renal toxicity of commercial formulation of roundup herbicide and glyphosate alone in male albino rat. International Journal of Toxicology 37 (4):285-95.

El-Shenawy NS, 2009. Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. Environmental Toxicology and Pharmacology 28**:** 379-385.

Harish SR and Murugan K, 2011. Oxidative stress indices in natural populations of Avicennia alba Blume as biomarker of environmental pollution. Environmental Research 111: 1070-1073.

Hou YJ, Zhao YY, Xiong B, Cui XS, Kim NH, Xu YX and Sun SC, 2013. Mycotoxin containing diet causes oxidative stress in the mouse. PloS One 8 (3): e60374.

Howe CM, Berrill M, Pauli DB, Helbing CC, Werr K and Veldhoen N, 2004. Toxicity of glyphosate-based pesticides to four North American frog

species. Environmental Toxicology and Chemistry 23: 1928–1938.

Ikpeme EV, Udensi O, Ekaluo UB and Solomon TO, 2012. Efficacy of ascorbic acid in reducing glyphosate induced toxicity in rats. British Biotechnology Journal 2 (3): 157-168.

Jasper R, Locatelli GO, Pilati C and Locatelli C, 2012. Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup®. Interdisciplinary Toxicology 5 (3): 133-140.

Karimi JH, Novin L and Poor Ahmadi M, 2014. Effect of the herbicide glyphosate on renal tissues in adult female rats. Journal of Jahrom University of Medical Sciences 11 (4): 10.

Kremer RJ and Means NE, 2009. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. European Journal of Agronomy 31 (3): 153-161.

Kubota K and Wakana A, 2011. Sample-size formula for case-cohort studies. Epidemiology 22 (2): 279.

Lakshman M, 2017. Diagnostic Electron Microscopy (EM) for Avian Diseases-An Overview. International Journal of Science and Research 6: 1478-1483.

Lakshman M, 2019. Application of conventional electron microscopy in aquatic animal disease diagnosis: A review. Journal of Entomology and Zoology Studies 7: 470-475.

Lall SB, Das N, Rama R, Peshin SS, Khattar S, Gulati K and Seth SD, 1997. Cadmium induced nephrotoxicity in rats. Indian Journal of Experimental Biology 35 (2): 151-154.

Langeswaran K, Jagadeesan AJ and Balasubramanian MP, 2012. Modulation of membrane bound ATPases and metabolizing enzymes against n-nitosodiethylamine (DEN) induced primary liver cancer in wistar albino rats. International Journal of Pharmacology and Biological Sciences 3 (2):156-65.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, 1951. Measurement of protein with the Folin phenol reagent. Journal of Biological Chemistry 193 (1): 265-275.

Luna GLHT, 1968. Manual of histological and special staining techniques. 2nd ed: The Blakistone Division McGraw-Hill Book Company, Inc. New York, Toronto London: 1-5 and 9-34.

Madesh M and Balasubramanian KA, 1998. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian Journal of Biochemistry and Biophysics 35: 184-188.

Moron MS, Depierre JW and Mannervik B, 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta (BBA)-General Subjects 582: 67-78.

Namratha ML, Lakshman M, Jeevanalatha M, Anil Kumar B, 2019. Effect of Glyphosate (GLP) Induced Toxicity on Body Weights and Gross Pathology: Ameliorative Effect of Ascorbic Acid (AA) in Wistar Rats. International Journal of Current Microbiology and Applied Sciences 8: 1486-1493.

Nieto FJ, Iribarren C, Gross MD, Comstock GW and Cutler RG, 2000. Uric acid and serum antioxidant capacity: a reaction to atherosclerosis. Atherosclerosis 148 (1): 131-139.

Santos JB, Ferreira EA, Kasuya MCM, Silva AA and Procopio SO, 2005. Tolerance of Bradyrhizobium strains to glyphosate formulations. Crop Protection 24: 543–547.

Scandalios JG, 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Brazilian Journal of Medical and Biological Research 38: 995–1014.

Snedecor GW and Cochran G, 1994. Statistical methods, 8th ed, IOWA State University Press, Amer, IOWA, USA.

Sun F, Hamagawa E, Tsutsui C, Ono Y, Ogiri Y and Kojo S, 2001. Evaluation of oxidative stress during apoptosis and necrosis caused by carbon tetrachloride in rat liver. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1535 (2): 186-191.

Tang J, Hu P, Li Y, Win-Shwe TT and Li C, 2017. Ion imbalance is involved in the mechanisms of liver oxidative damage in rats exposed to glyphosate. Frontiers in Physiology 8: 1083.

Tizhe EV, Ibrahim NDG, Fatihu MY, Onyebuchi II, George BDJ, Ambali SF and Shallangwa JM, 2014. Influence of zinc supplementation on histopathological changes in the stomach, liver, kidney, brain, pancreas and spleen during subchronic exposure of Wistar rats to glyphosate. Comparative Clinical Pathology 23 (5): 1535-1543.

Weir CJ, Muir SW, Walters MR and Lees KR, 2003. Serum urate as an independent predictor of poor outcome and future vascular events after acute stroke. Stroke 34 (8): 1951-1956.

Williams GM, Kroes R and Munro IC, 2000. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regulatory Toxicology and Pharmacology 31 (2): 117–65.

Wunnapuk K, Gobe G, Endre Z, Peake P, Grice JE, Roberts MS, Buckley NA and Liu X, 2014. Use of a glyphosate-based herbicide-induced nephrotoxicity model to investigate a panel of kidney injury biomarkers. Toxicology Letters 225 (1):192-200.

Youness ER, Agha FE, El-Toukhy SE, El-Naggar SM, Selim AA and Ibrahim AM, 2016. The protective effect of orange juice on glyphosate toxicity in adult male mice. Journal of Chemical and Pharmaceutical Research 8 (3): 13-28.