Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2021.021

RESEARCH ARTICLE

Assessment of Vitamin C Protective Activity in Glyphosate-Induced Hepatotoxicity in Rats

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

[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
*Corresponding author: mekala_bry@yahoo.com

ARTICLE HISTORY (20-206)

Received: April 17, 2020
Revised: November 20, 2020
Accepted: November 21, 2020
Published online: February 17, 2021

Key words:
Glyphosate
Histopathology
Oxidative stress
Rats
Serum biochemistry
Vitamin C

ABSTRACT

Glyphosate is widely used herbicide throughout the world that is posing a major threat to human and animal life. The clinical outcomes of ingesting the herbicide include hepatotoxicity. Vitamin C is a known antioxidant compound that prevents free radical induced cellular damage by terminating the chain reactions. The aim of the current study was to assess the protective role of vitamin C (250 mg/kg BW) against glyphosate induced hepatotoxicity at a dose of 500 mg/kg BW (1/10 LD_{50}) in male albino Wistar rats. The animals were treated orally, daily for three weeks. The serum biochemical assays (aspartate transaminase, alanine transaminase, alkaline phosphatase and total protein), tissue oxidative stress parameters, histopathology and ultrastructure pathology of liver were studied. Oxidative stress in liver tissue was monitored by determining the levels of malondialdehyde content, reduced glutathione and superoxide dismutase. Histopathology and ultrastructure pathology of liver sections were studied. There were significant alterations in the above parameters in glyphosate treated rats. However, administration of vitamin C caused a mild to moderate ameliorative effect on the parameters investigated.

To Cite This Article: Namratha ML, M Lakshman, M Jeevanalatha and Kumar BA, 2021. Assessment of vitamin c protective activity in glyphosate-induced hepatotoxicity in rats. Pak Vet J, 41(3): 439-445. http://dx.doi.org/10.29261/pakvetj/2021.021

INTRODUCTION

Glyphosate (GLP) is the active ingredient of Roundup® was first sold commercially by Monsanto in 1974. It is marketed as a non-selective, broad spectrum, post emergence herbicide used to control weeds in emerged grasses, pastures, rice, corn and soy plantations (Smith and Oehme, 1992). Glyphosate was highly accepted by the tea planters in the past two decades. It has a very good market size in the tea sector of West Bengal and Assam (Choudhury *et al.,* 2016). The farmers and animals are being exposed directly and indirectly during its application and disposal of containers leading to fatalities. These herbicides may contaminate the soil and ground water, thereby they may find the way to enter into the crops (commercial and food crops). These crop residues and by-products usually are being used as major ingredients in animal feed (Dallegrave *et al.*, 2007).

In addition to the glyphosate salts, commercial formulations of GLP contain additives such as surfactants (Polyethoxylated tallow amine - POEA), which are added to the GLP to enable it to wet the leaves and penetrate the cuticle of plants (Franz *et al.,* 1997). In this context, Mesnage *et al*. (2013) revealed that POEA was one of the active ingredients of Roundup formulations inducing human toxicity, triggering necrosis by disrupting cell membranes around its critical micellar concentration, rather than glyphosate, that leads to apoptosis.

Many pollutants can induce damage in biological systems, including the mammalian liver, which is the chief site for detoxification and biotransformation processes. These involve formation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), the superoxide anion (O^{2-}), and the hydroxyl radical (·OH) (Ahmad *et al*., 2000; Harish and Murugan, 2011). Glyphosate exposure and metabolism in the liver of mice led to an excessive production of malondialdehyde (MDA) and oxidative stress through unregulated generation of ROS (Cavusoglu *et al.,* 2011 and Youness *et al.,* 2016). It also causes significant alterations in

hematological and serum biochemical parameters (Jasper *et al.,* 2012). Gastrointestinal corrosive effects, with mouth, throat, epigastric pain and dysphagia are common. Renal and hepatic impairments are also frequent and usually reflect reduced organ perfusion. In another study, it was shown that glyphosate causes liver damage in rats, by the leakage of intracellular liver enzymes (Benedetti *et al.,* 2004). In many studies, an association between glyphosate use and the risk of non-Hodgkin's lymphoma was suggested (Zhang *et al.,* 2019). The GLP being an endocrine disruptor in human cells induces DNA damage and interferes with DNA repair machinery, leading to genomic instability and cancer development. The toxic effects of GLP could be due to uncoupling of oxidative phosphorylation (Ikpeme *et al*., 2012).

On the other hand, vitamin C is a naturally occurring organic compound with antioxidant properties, found both in animals and plants. It functions as a redox buffer which can reduce, and thereby neutralize ROS (Pehlivan, 2017). Being an antioxidant, it is involved in the prevention of cellular damage by safely interacting with free radicals and terminating the chain reactions before vital molecules are damaged. It also removes free radical intermediates and inhibits other oxidative reactions. Possibly, the administration of GLP reacts with host tissue and releases free radicals into the systems, which might have an invariable action on organs like liver and kidneys for its detoxification and excretion. Treating with vitamin C in glyphosate toxicity might have mopped up the released free radicals, thus maintaining the integrity of the cells (Ikpeme *et al.,* 2012).

Given the increasing use of glyphosate-Roundup®, along with the lack of information on its toxicity in mammals, the current study was designed to evaluate the protective role of vitamin C against glyphosate induced hepatotoxicity from serum biochemical, oxidative stress parameters, histopathology and ultrastructure pathology, using male albino Wistar rats for a period of three weeks.

MATERIALS AND METHODS

Chemicals: Commercial glyphosate formulation Roundup® was obtained from Seed Research and Technology Centre (SRTC), Professor Jayashankar Telangana State Agriculture University (PJTSAU), Hyderabad-30, which contains 41% glyphosate as the active ingredient and vitamin C was obtained from S.D. Fine-Chem Ltd., Mumbai, India.

Experimental animals: Clinically healthy adult male albino Wistar rats weighing 200-240 g, were obtained from Jeeva Life Sciences (ISO 9001:2015 certified company), Hyderabad. The animals were acclimatized at 25±2°C and 12 h of a light- dark cycle for 7 days. All the rats were provided with standard pellet diet (low fat and nutritionally balanced food) and deionized drinking water *ad libitum* throughout the experimental period. The experiment was carried out according to the guidelines and prior approval of Institutional Animal Ethics Committee (IAEC-No.01-2019).

Experimental design: Forty-eight male rats were randomly divided into four groups named as G1-G4 consisting of twelve (12) animals each. G1 served as control received distilled water, G2 received GLP (500 mg/kg BW), G3 received vitamin C (250 mg/kg BW) and G4 received GLP + vitamin C (500 mg/kg BW + 250 mg/kg BW). The dose regimens were administered *per os* once daily for a period of three weeks. Six rats from each group were sacrificed on 7th and 21st day of experiment using carbon dioxide chamber.

Biochemical evaluation: Just before sacrifice, 1-1.5 mL of blood was collected from retro-orbital plexus with the help of capillary tube (3mm) into non EDTA vacutainers to separate serum for biochemical estimations into eppendorf tubes. All the biochemical parameters *viz.,* AST, ALT, ALP and TP were analyzed using Erba Mannheim Diagnostic kits obtained from Erba Diagnostics, Hyderabad, India with automated serum analyzer (Star 21 Plus, Rapid Diagnostic Pvt. Ltd.).

Oxidative stress indices: One gram of liver sample with 10 mL of 0.2 M Tris HCl buffer (pH 7.2) was taken into a tissue homogenizer to get 10 per cent homogenate to carry out all the tissue antioxidant profiles. The tissue oxidation was measured by the reaction of the lipid peroxidation end products like MDA (Balasubramanian *et al.,* 1988), tissue protein (Lowry *et al.,* 1951), reduced glutathione (GSH) (Moron *et al.,* 1979) and activity of superoxide dismutase (SOD) (Madesh and Balasubramanian, 1998) to know the antioxidant status.

Histopathological studies: The tissue samples of liver were collected and fixed in 10 % Neutral Buffered Formalin (NBF) soon after necropsy. The samples were processed, sectioned (5 µm thick) and stained with Hematoxylin and Eosin (H&E) for histopathological examination as per the standard procedure (Luna, 1968).

Ultrastructure Pathology: Soon after sacrifice thin slices of liver was fixed in 2.5% glutaraldehyde (PBS based) and processed for transmission electron microscopy (TEM) and scanning electron microscopy (SEM) as per standard protocol (Lakshman, 2017 and 2019).

Statistical analysis: Data obtained were subjected to statistical analysis by applying one way ANOVA using Statistical Package for Social Sciences (SPSS) version 16.0. Differences between the means were tested by using Duncan's multiple comparison tests and significance level was set at $P<0.05$ (Snedecor and Cochran, 1994).

RESULTS

Biochemical results: The serum biochemical assays showed a significant ($P<0.05$) increase in the mean values of AST, ALT, ALP and TP in GLP treated rats (G2) and ameliorative group (G4) as compared to control (G1) on 7th and 21st day of experiment. No significant difference was noticed between the groups 1 and 3. The serum AST and ALP activities in vitamin C treated group (G4) were significantly reduced in comparison to group 2. No significant difference was noticed in the mean values of ALT and TP except on 21st day as compared to group 2. However, the values were significantly high when compared to control group. (Table 1).

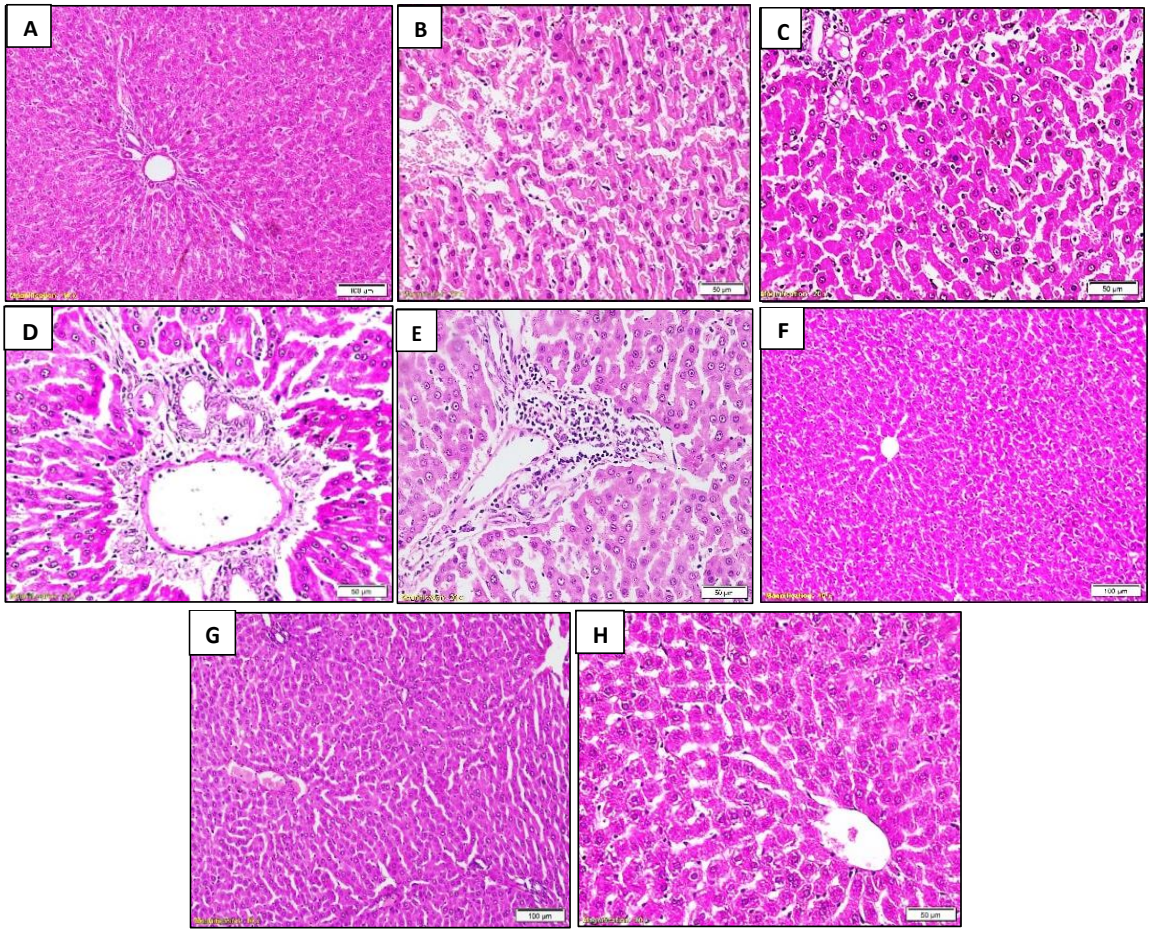

Fig.1: Photomicrograph of histopathology of liver: A: Liver of rat from G1 showing normal portal triad and radiating hepatic cords (X 100): B: Liver of G2 rat on day 7 showing dilated sinusoids with blood cells, necrosis of hepatocytes and pyknotic nuclei (X 200): C: Liver of G2 rat on day 7 showing mild edema, Kupffer cell proliferation, sinusoidal hemorrhages and MNC infiltration (X 200): D: Liver of G2 rat on day 21 showing periportal necrosis, distorted cords, increased number of Kupffer cells, round cell proliferation, vacuolar degeneration and rhectic nuclei (X 200): E: Liver of G2 rat on day 21 showing perivascular necrosis, infiltration of round cells, distorted cords and necrosis of hepatocytes with rhectic nuclei (X 200): F: Liver of G3 rat showing normal central vein and radiating hepatic cords (X 100): G: Liver of G4 rat on day 7 showing mild regeneration of hepatic cords with central vein congestion (X 100): H: Liver of G4 rat on day 21 showing central vein dilation and vacuolar degeneration with dilated sinusoids (X 200). All sections were stained with H&E

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

Parameter	Control		Glyphosate		Vitamin C		Glyphosate+Vit C	
	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21
Aspartate Transaminase	36.24±1.22^{a}	38.87±2.12^{a}	59.66±2.18^{c}	71.83±1.86^{c}	34.2±1.85^{a}	38.03±2.94^{a}	50.16±1.85^{b}	63.5±1.52^{b}
Alanine Transaminase	35.08±3.28^{a}	34.99±2.93^{a}	53.74±2.88^{b}	63.92±3.21^{b}	35.66±2.29^{a}	34.33±3.63^{a}	48.81±3.11^{b}	56±2.84^{b}
Alkaline Phosphatase	83.33±1.54^{a}	88.5±1.94^{a}	122±2.52^{c}	135.5±1.97^{c}	81.33±2.37^{a}	89.66±1.28^{a}	106±2.14^{b}	115.5±2.64^{b}
Total Proteins	4.4±0.09^{a}	4.61±0.07^{a}	5.76±0.31^{b}	6.75±0.04^{c}	4.55±0.08^{a}	4.46±0.04^{a}	5.45±0.42^{b}	6.48±0.11^{b}
Malondialdehyde	3.53±0.72^{a}	3.79±0.03^{a}	7.16±0.18^{c}	8.08±0.14^{d}	3.47±0.72^{a}	3.20±0.66^{a}	6.43±0.16^{b}	7.34±0.99^{c}
Reduced Glutathione	10.33±0.07^{c}	10.58±0.09^{c}	8.36±0.1^{a}	7.28±0.08^{a}	10.51±0.05^{c}	10.73±0.03^{c}	8.77±0.04^{b}	8.48±0.17^{b}
Superoxide Dismutase	11.34±0.27^{c}	11.57±0.11^{c}	9.76±0.15^{a}	8.49±0.1^{a}	11.57±0.16^{c}	11.8±0.09^{c}	10.71±0.11^{b}	9.6±0.14^{b}

Values are Mean±SEM (n=6); One-way ANOVA: Means with different superscripts in a column differ significantly at P<0.05 (*).

Oxidative stress indices: Studies on tissue oxidative stress parameters of liver showed a significant (P<0.05) elevation in mean values of TBARS in glyphosate treated group (G2) as compared to the control group (G1). Supplementation of vitamin C combined with glyphosate (G4), showed a moderate reduction in levels of MDA significantly on 7th and 21st day of experiment when compared to group 2. However, the values were significantly increased when compared to control group. No significant difference was noticed between the groups 1 and 3.

The mean values of reduced GSH and SOD were significantly (P<0.05) declined in glyphosate treated group on 7th and 21st day of experiment when compared to control group. In ameliorative group (G4), a significant increase in the levels of reduced GSH and SOD were observed as compared to glyphosate treated group (G2) on 7th and 21st day of experiment. However, the values were significantly lower when compared to control group (G1). No significant difference was noticed in group 3 when compared to control group on 7th and 21st day of experiment. (Table 1).

Histopathological results: Liver sections of control group (G1) showed normal appearance of the portal triad and hepatocytes arranged in linear cords (Fig. 1A). Different sections of liver from GLP treated group (G2) on 7th day revealed necrotic hepatocytes, dilated sinusoids and sinusoidal hemorrhages (Fig. 1B). Few other sections also showed mild edema with mild Kupffer cell proliferation and mononuclear cell infiltration (MNC)

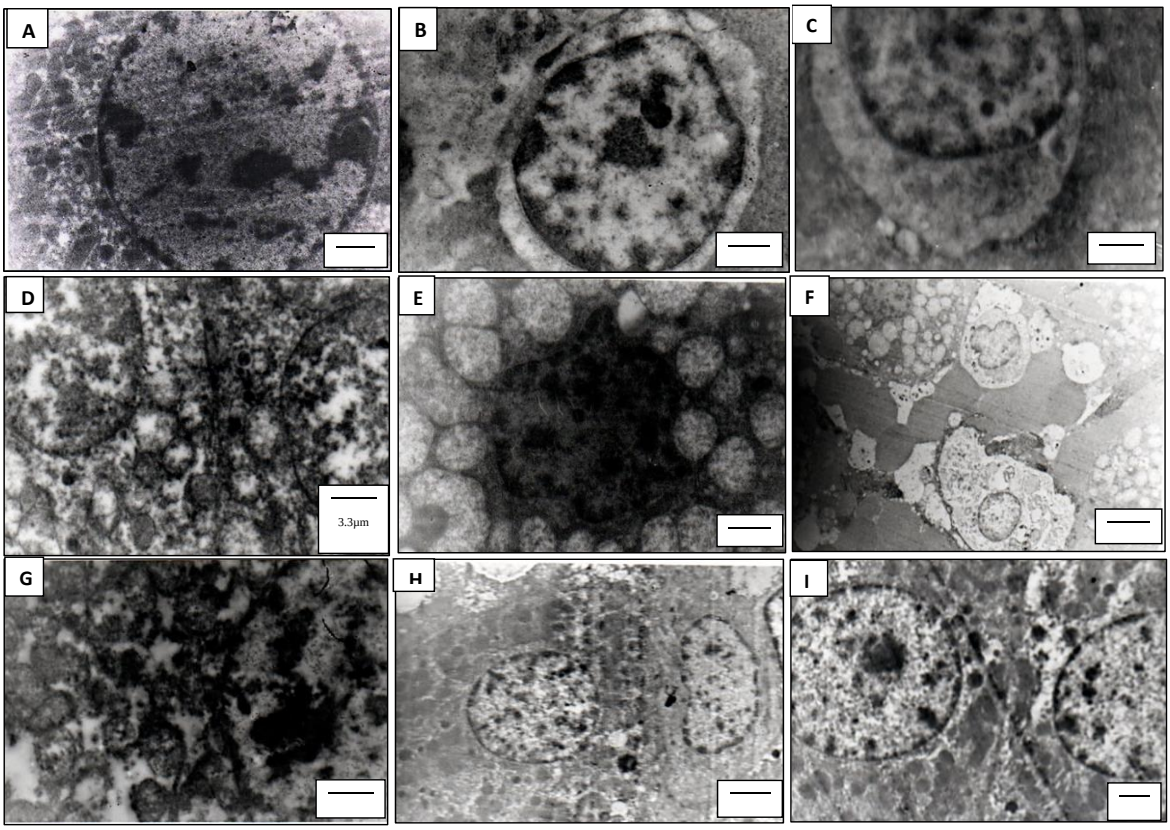

Fig. 2: TEM of liver: A: Liver of rat from G1 showing hepatocyte with normal nucleus, nucleolus and uniform mitochondria (9650X): B: Liver of rat from G2 on day 7 showing apoptotic nuclei, amorphous appearance of cytosol and indistinct cell organelles (11580X): C: Liver of rat from G2 on day 7 showing few vacuolated areas and condensed nucleus with mild margination of chromatin (11580X): D: Liver of rat from G2 on day 7 showing loose cell junctions with vacuolar cytosol, condensed nucleus and vesicular mitochondria (5790X): E: Liver of rat from G2 on day 21 showing swollen and vacuolar mitochondria in cytosol with deformed nucleus (9650X): F: Liver of rat from G2 on day 21 showing abnormal hepatocytes with vacuolar mitochondria, dilated micro capillary and dilated sinusoids (2316X): G: Liver of rat from G3 showing electron dense granular uniform mitochondria and thick nuclear membrane (11580X): H: Liver of rat from G4 on day 7 showing mild regeneration, normal nuclei and Kupffer cell (3860X): I: Liver of rat from G4 on day 21 showing loose intercellular junctions, mild margination of chromatin, dilated SER, electron dense amorphous mitochondria and thick nuclear membrane with dilated nuclear pores (4825X). All sections were stained with uranyl acetate and lead citrate.

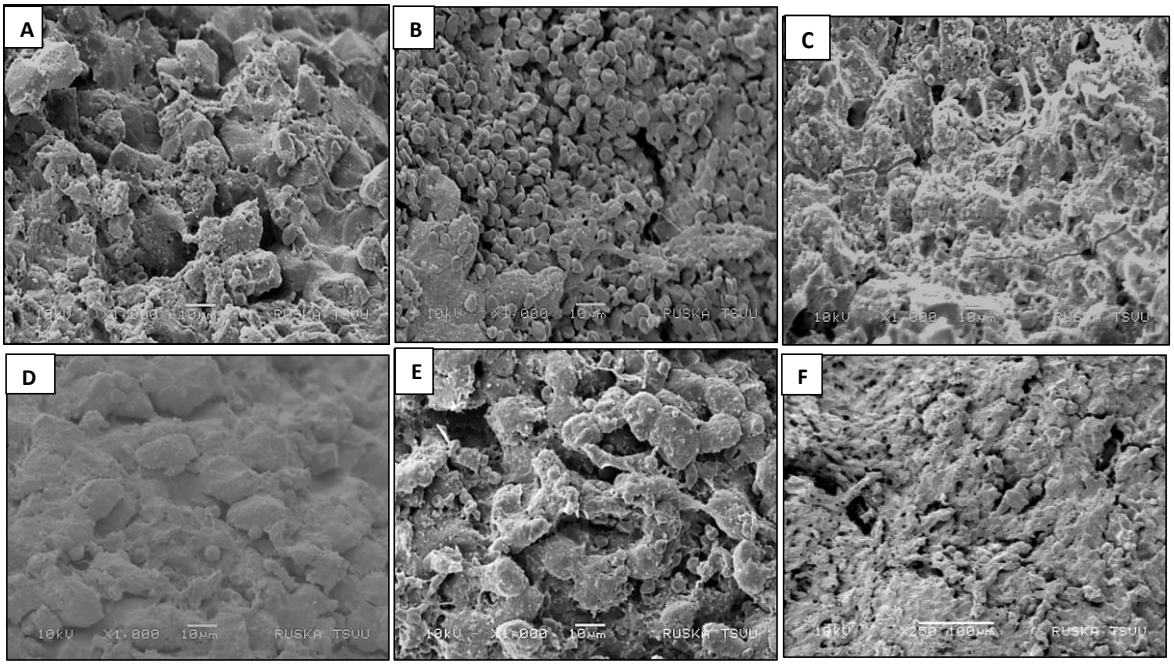

Fig. 3: SEM of liver: A: Liver section of rat from G1 showing normal hepatocytes: B: Liver section of rat from G1 on day 7 showing dilated sinusoids, sinusoidal hemorrhage and mild fibrous tissue proliferation: C: Liver section of rat from G1 on day 21 showing distorted hepatocytes with few blood cells: D: Liver section of rat from G3 showing intact hepatocytes: E: Liver section of rat from G4 on day 7 showing regeneration of hepatocytes with few blood cells: F: Liver section of rat from G4 on day 21 showing rearrangement of cords with dilation of sinusoids.

(Fig. 1C). Sections of liver on 21st day showed severe and progressive lesions when compared to 7th day periportal necrosis, distorted cords, increased number of Kupffer cells, round cell proliferation, vacuolar degeneration and rhectic nuclei (Fig. 1D). Few sections revealed perivascular necrosis, infiltration of round cells and distorted cords (Fig. 1E). Liver sections of group 3 rats showed normal appearance of hepatic lobules consisting of a central vein (CV) and radiating hepatocytes (Fig. 1F). The liver sections of vitamin C administered group (G4) showed comparatively low level of lesions than GLP treated group (G2).

Majority of the sections showed mild regeneration of hepatic cords with congestion of CV on 7th day (Fig. 1G). Dilated sinusoids, CV congestion and necrotic hepatocytes with karyorrhectic nuclei were noticed on 21st day post treatment (Fig. 1H).

Ultrastructure pathology- TEM: Ultrathin sections of liver from group 1 on 21st day revealed intact hepatocytes with normal nucleus, nucleolus and uniform mitochondria (Fig. 2A). The TEM of liver sections from G2 on 7th day revealed apoptotic nuclei (moderate to severe margination of chromatin), amorphous appearance of cytosol and indistinct cell organelles (Fig. 2B). Some sections also showed vacuolated areas, condensed nucleus with mild margination of chromatin (apoptotic nuclei), loose cell junctions with vacuolar cytosol, condensed nucleus and vesicular mitochondria (Fig. 2C and 2D). On 21st day, liver sections showed swollen and vacuolar mitochondria in cytosol with deformed nucleus, dilated micro capillaries were noticed (Fig. 2E and 2F). Group 3 liver sections on 21st day revealed electron dense granular uniform mitochondria and thick nuclear membrane (Fig. 2G). Liver sections from G4 on 7th day revealed mild regeneration of hepatocytes, normal nuclei and Kupffer cells (Fig. 2H). On 21st day, loose intercellular junctions, mild margination of chromatin, dilated smooth endoplasmic reticulum (SER), electron dense amorphous mitochondria and thick nuclear membrane with dilated nuclear pores were noted (Fig. 2I).

SEM: SEM of liver from G1 showed intact hepatocytes and normal architectural details. (Fig. 3A). The surface area of group 2 liver on 7th day revealed dilated sinusoids, distorted hepatic cords, sinusoidal hemorrhages and mild fibrous tissue proliferation (Fig. 3B). On 21st day, liver samples revealed distorted hepatocytes with few blood cells (Fig. 3C). G3 liver slices showed intact hepatocytes (Fig. 3D). Mild regeneration of hepatocytes and reconstruction of cords were noticed in G4 liver on 7th day (Fig. 3E). On 21st day, there was rearrangement of cords with dilation of sinusoids (Fig. 3F).

DISCUSSION

The current work revealed that rats exposed to glyphosate displayed a pronounced impairment in liver function which was confirmed by histopathological and ultrastructural alterations. Rats administered with glyphosate showed various clinical signs as anorexia, decreased water intake, dullness, mild diarrhea and weakness. No mortalities were recorded during the study.

The results showed that glyphosate is hepatotoxic which is confirmed in our study by the major alterations noticed in the serum biochemical assays and oxidative stress indices. High levels of transaminases (AST and ALT) in serum can be a sign of liver damage and disruption of normal liver function. In our study, a steady increase in the levels of serum enzymes were noticed on 7th and 21st day of experiment in GLP treated group which could be attributed to the damage caused by GLP on membrane permeability of hepatocytes leading to the leakage of these enzymes from distorted hepatocyte cytosol into the bloodstream. These alterations are related to decreased glutathione level and increased lipoperoxidation in the hepatic tissue. In the present study, the increase in transaminase levels have been supported by the pathological findings, characterized by fibrosis in the portal area, necrosis of hepatocytes and Kupffer cell proliferation. Similar increase in the levels of transaminases post treatment with glyphosate have been reported by El-Shenawy (2009), Cavusoglu *et al*. (2011), Jasper *et al*. (2012) and Tizhe *et al.* (2014). Benedetti *et al.* (2004) revealed that oral exposure of male Wistar rats to glyphosate-Biocarb® for a period of 75 days led to increased levels of transaminases (ALT and AST) and induced cellular alterations, as shown by an increase of connective tissue and deposition of collagen in liver cells. Similar findings were observed in the present study, when rats were treated with glyphosate- Roundup® for three weeks. The excessive production of free radicals and lipid peroxides might cause an alteration of membrane permeability leading to the leakage of these cytosolic enzymes from the hepatic tissue. Soudani *et al.* (2019) reported administration of quercetin to glyphosate treated rats, significantly reduced transaminase activities in plasma, suggesting that quercetin probably prevented the leakage of these marker enzymes by keeping the structural integrity of the liver. Tizhe *et al.* (2014) reported that supplementation with zinc brought about restoration of the AST activity in rats, indicating its antioxidant activity against glyphosate induced hepatotoxicity. Administration of *Ginkgo biloba* extract counteracted the toxic effects of glyphosate, thereby restoring the levels of serum enzymes (Cavusoglu *et al*., 2011). Youness *et al.* (2016) reported that administration of orange juice at a dose of 30 mL/kg caused reduction in the levels of AST and ALT in glyphosate treated group indicating the antioxidant role of orange juice. In the present study, a significant reduction in the mean values of serum enzymes in vitamin C treated group (G4), indicates the potent antioxidant ability of Vit. C which safely interacts with the free radicals and terminates the chain reactions before vital molecules are damaged. It also eliminates free radical intermediates and impedes other oxidative reactions (Ikpeme *et al.*, 2012). The elevated levels of ALP in the present study may be attributed due to biliary damage. Contrary to this, Caglar and Kolankaya (2008) observed a decrease in serum levels of AST and ALT in rats exposed to GLP (56 and 560 mg/kg BW) during 5th and 13th week of their experiment. The variation in biochemical observation perhaps could be due to the dose and duration of exposure.

A significant increase in the mean values of TP were recorded in groups 2 and 4 as compared to group 1 on 7th

and 21st day of experiment which might be due to damage of hepatic parenchyma leading to the leakage of proteins from the cytosol into bloodstream. Contrary to this, El-Shenawy (2009) and Tizhe *et al*. (2014) reported no change in the levels of TP.

Oxidative stress refers to the oxidation and anti-oxidation imbalance *in vivo* (Hou *et al.,* 2013). When the defense systems are insufficient for neutralizing the Reactive oxygen species (ROS), oxidative damage can occur and one of the most serious types of which is membrane lipid peroxidation (LPO). Our study showed a significant increase in the levels of MDA in liver tissue of GLP treated rats which could be due to increased LPO leading to an increased intracellular accumulation of ROS thereby leading to injury of organs. Vitamin C serves dual role of a pro-oxidant and an antioxidant (Pehlivan, 2017). Supplementation of vitamin C decreased the MDA levels significantly indicating the antioxidant property of vitamin C. Cavusoglu *et al.* (2011) showed that treatment of Swiss albino mice with *Gingko biloba* leaf extract (150 mg/ kg BW) produced an improvement in indices of hepatotoxicity, nephrotoxicity, lipid peroxidation, and genotoxicity induced by glyphosate (50 mg/kg BW). These *in vivo* results showed that *Ginkgo biloba* may present a significant protective effect against the toxicity induced by glyphosate. Youness *et al*. (2016) reported an elevated level of MDA in the liver tissue which could be due to oxidative stress by the production of free radicals which affects the integrity of cells by damage of lipids, proteins and DNA. Jasper *et al.* (2012) and Tang *et al.* (2017) also reported an increase in levels of liver MDA post treatment with glyphosate as reported in the present study. The association of Roundup with antioxidants such as N-acetyl-L-cysteine (NAC), vitamins C and E, could reduce the toxic damage. Being an antioxidant, Vit. C safely interacts with the free radicals and terminates the chain reactions before vital molecules are damaged. It also eliminates free radical intermediates and impedes other oxidative reactions (Ikpeme *et al.,* 2012). It could be assumed that Roundup, by lowering the levels of GSH and SOD led to an oxidative imbalance and induced oxidative processes, resulting in increased cell death. The depletion of hepatic GSH indicates the activation of antioxidant defenses, probably due to increased hydrogen peroxide generation (El-Shenawy, 2009). A significant decline in the values of GSH in the present study might be due to the ability of GLP to induce mitochondrial damage and subsequently leading to an increased ROS production which is responsible for oxidative stress, these results are further correlated with histopathology and ultrastructure pathology. However, there was a significant increase in GSH levels in vitamin C treated rats along with GLP signifying the ameliorative action of Vit. C to some degree. Hepatic SOD activity is also a molecular indicator for liver damage (Li *et al.,* 2013). Decreased activities of GSH and SOD and increased levels of MDA in the liver tissue in the present study were indicative of oxidative stress induced by GLP. Group 4 rats showed mild improvement compared to group 2 indicating the the antioxidant effect of Vit. C and its ability to act as a redox buffer capable of neutralizing the reactive oxygen species induced by the GLP. It would be necessary to perform studies *in vivo* with Roundup® plus different antioxidants or combination of antioxidants to confirm either a protective effect or treatment potential against Roundup® intoxication.

Grossly, severe congestion of liver was reported after treatment with glyphosate (500 mg/kg BW) as a result of vascular changes (Namratha *et al.,* 2019). Reduction in absolute weights of liver was reported post treatment with glyphosate due to the major pathological changes observed in the hepatocytes including degeneration, necrosis and leakage of enzymes which adversely affected synthesis and utilization of proteins (Namratha *et al.,* 2019). The histopathological results are in line with the biochemical and oxidative stress results. Benedetti *et al.* (2004) reported that these changes could have contributed to the impairment of regular function of the liver due to xenobiotics modification in the detoxification processes. Çaglar and Kolankaya (2008) also reported similar lesions in the liver post treatment with glyphosate (560 mg/kg BW) as liver being the major site for detoxification and metabolism of chemicals. Because of this, there is severe injury in liver tissue. In the present study, these changes could be due to the GLP induced molecular cell injury to the hepatocytes. Hypothetically, the degenerated and necrotic hepatocytes initiated lymphoid aggregation as a process of defense mechanism. Pretreatment with vitamin C markedly improved the histological structure of the liver cell membrane and decreased the release of aminotransferases from hepatocytes, in addition to decline in the oxidative stress markers. Administration of Vit. C to glyphosate treated rats was quite appreciable as it reduced the histological alterations to some degree. This effect could be attributed to the antioxidant and free radical scavenging properties of vitamin C, which considerably reduced the oxidative insult leading to the reduction of pathological changes and restoration of normal physiological functions.

Szarek *et al.* (2000) reported the presence of myelin like structures in the cytoplasm of carp hepatocytes and swollen mitochondria suggesting that despite low toxicity of glyphosate to fish, its low rate of dissipation/degradation in water may induce toxic effects shortly after application. Hypothetically, in the present study the ultrastructural changes might have been triggered by GLP leading to the generation of ROS consequently mitochondrial structures were damaged, thereby causing leakage of cytochrome C and activation of apoptotic death pathways which is supported by biochemical, oxidative stress indices and histopathological findings. The alterations noted in the nucleus and subcellular structures infers that glyphosate is capable of initiating the apoptotic death pathways. Liver sections from group 4 revealed mild regeneration. These changes are attributed to the toxic effect of GLP despite the antioxidant property of vitamin C.

Conclusions: The present study suggests that the GLP intoxication can cause severe free radical injury and apoptosis. Alteration in the serum biochemical assays, oxidative stress indices, histopathological and ultrastructural findings can conclude the potential hepatotoxic effect of GLP. Administration of vitamin C alone has not completely ameliorated the toxic changes

induced by GLP despite of its high antioxidant properties. There is also a scope to study the effective ameliorative agent or combination of different agents which can neutralize the toxic action of GLP.

Authors contribution: ML, MJ, BAK designed the experiment. MLN run all experiments, statistical analysis and drafted the manuscript. ML, interpreted histological results and corrected the manuscript. All authors read and approved the final manuscript.

REFERENCES

Ahmad I, Hamid T, Fatima M, *et al.,* 2000. Induction of hepatic antioxidants in freshwater catfish (Channa punctatus Bloch) is a biomarker of paper mill effluent exposure. Biochim. Biophys. Acta 1523:37-48.

Balasubramanian KA, Manohar M and Mathan VI, 1988. An unidentified inhibitor of lipid peroxidation in intestinal mucosa. Biochim Biophys Acta 962:51-8.

Benedetti AL, de Lourdes Vituri C, Trentin AG, *et al.,* 2004. The effects of subchronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb®. Toxicol Lett 153:227-32.

Çaglar S and Kolankaya, 2008. The effect of sub- acute and sub- chronic exposure of rats to the glyphosate- based herbicide Roundup. Environ Toxicol Pharmacol 25:57-62.

Cavusoglu K, Yapar K, Oruc E, *et al.,* 2011. Protective effect of Ginkgo biloba L. leaf extract against glyphosate toxicity in Swiss albino mice. J Med Food 14:1263-72.

Choudhury PP, Singh R, Ghosh D, *et al.,* 2016. Herbicide use in Indian agriculture. ICAR-Directorate of Weed Research, Jabalpur, Madhya Pradesh.

Dallegrave E, Mantese FD, Oliveira RT, *et al.,* 2007. Pre-and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Arch Toxicol 81:665-73.

El-Shenawy NS, 2009. Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. Environ Toxicol Pharmacol 28:379-85.

Franz JE, Mao MK and Sikorski JA, 1997. Uptake, transport and metabolism of glyphosate in plants. Glyphosate: a unique global herbicide. American Chemical Society, Washington DC pp:143-81.

Harish SR and Murugan K, 2011. Oxidative stress indices in natural populations of Avicennia alba Blume. as biomarker of environmental pollution. Environ Res 111:1070-3.

Hou YJ, Zhao YY, Xiong B, *et al.,* 2013. Mycotoxin containing diet causes oxidative stress in the mouse. PloS One 8:e60374.

Ikpeme EV, Udensi O, Ekaluo UB *et al.,* 2012. Efficacy of ascorbic acid in reducing glyphosate induced toxicity in rats. Br Biotechnol J 2:157-68.

Jasper R, Locatelli GO, Pilati C *et al.,* 2012. Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup®. Interdiscipl Toxicol 5:133-40.

Lakshman M, 2017. Diagnostic Electron Microscopy (EM) for Avian Diseases-An Overview. International J of Sci Res 6:1478-83.

Lakshman M, 2019. Application of conventional electron microscopy in aquatic animal disease diagnosis: A review 7:470-5.

Li CM, Li L, Bai JY, *et al.,* 2013. Correlation between heat shock protein 32 and chronic heat induced liver injury in developing mice. J Therm Biol 38:513-9.

Lowry OH, Rosebrough NJ, Farr AL *et al.,* 1951. Measurement of protein with the Folin phenol reagent. J Biol Chem 193:265-75.

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

Madesh M and Balasubramanian KA, 1998. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J Biochem Biophys 35:184-8.

Mesnage R, Bernay B and Seralini, GE, 2013. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicol 313:122-8.

Moron MS, Depierre JW and Mannervik B, 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 582:67-78.

Namratha ML, Lakshman M, Jeevanalatha M, *et al.,* 2019. Effect of Glyphosate (GLP) on Organ Weights in Wistar Rats and its Amelioration with Ascorbic Acid. International J of Sci and Res 8:790-2.

Namratha ML, Lakshman M, Jeevanalatha M, *et al.,* 2019. Effect of glyphosate (GLP) induced toxicity on body weights and gross pathology: Ameliorative effect of Ascorbic Acid (AA) in Wistar rats. Int J Curr Microbiol App Sci 8:1486-93.

Pehlivan FE, 2017. Vitamin C: an antioxidant agent. Vitamin C Ed. Hamza, A. H. (In Tech), pp:24-32.

Smith EA and Oehme FW, 1992. The biological activity of glyphosate to plants and animals: a literature review. Vet Hum Toxicol 34:531-43.

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

Soudani N, Chaabane M, Ghorbel I, *et al.,* 2019. Glyphosate disrupts redox status and up-regulates metallothionein I and II genes expression in the liver of adult rats. Alleviation by quercetin. Gen Physiol Biophys 38:123-34.

Szarek J, Siwicki A, Andrzejewska A, *et al.,* 2000. Effects of the herbicide Roundup™ on the ultrastructural pattern of hepatocytes in carp (Cyprinus carpio). Mar Environ Res 50:263-6.

Tang J, Hu P, Li Y, *et al.,* 2017. Ion imbalance is involved in the mechanisms of liver oxidative damage in rats exposed to glyphosate. Front Physiol 8:1083.

Tizhe EV, Ibrahim NDG, Fatihu MY, *et al.,* 2014. Serum biochemical assessment of hepatic and renal functions of rats during oral exposure to glyphosate with zinc. Comp Clin Pathol 23:1043-50.

Youness ER, Agha FE, El-Toukhy SE, *et al.,* 2016. The protective effect of orange juice on glyphosate toxicity in adult male mice. J Chem Pharm Res 8:13-28.

Zhang L, Rana I, Shaffer RM, *et al.,* 2019. Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: a meta-analysis and supporting evidence. Mutat Res Rev Mutat Res 781:186-206.