Effect of Ethanolic Extract of *Malus domestica* Peel on Gentamicin-Induced Nephrotoxicity in Rats

Hema Kumari [1*], R. Aishwarya [2], R. Padmavathi [3]

[1]*Department of Pharmacology, G. Pulla Reddy College of Pharmacy, Hyderabad, Telangana, India-500028.*

[2]*Department of Pharmacology, G. Pulla Reddy College of Pharmacy, Hyderabad, Telangana, India-500028.*

[3]*Department of Pharmacology, G. Pulla Reddy College of Pharmacy, Hyderabad, Telangana, India-500028.*

**Corresponding author:* Hema Kumari

E-mail: hema263kumari@gmail.com

Abstract

Nephrotoxicity is particularly common since kidneys have the ability to excrete toxic substances, making kidney disorders a major issue of global proportions, however prevention methods are still being researched. The purpose of this study was to evaluate the effect of ethanolic extract of Malus domestica peel (MDPE) against gentamicin-induced nephrotoxicity in rats. Nephrotoxicity was induced in rats by gentamicin (80 mg/ kg/day., i.p) administration for 7 days. MDPE (100, 200 and 400 mg/kg/day., p.o) was administered for 28 days. The nephroprotective activity was evaluated by determining the levels of serum urea, uric acid and creatinine, renal MDA, SOD, CAT levels and body weight with histopathological studies. Gentamicin-induced nephrotoxicity was observed by a significant increase in the serum levels of urea, uric acid and creatinine, increased renal MDA levels and decreased renal SOD and CAT levels with multiple histological damage. Pre-treatment with MDPE, dose-dependently and significantly attenuated the changes caused by gentamicin administration. Findings of the study showed that MDPE has a nephroprotective effect against gentamicin-induced nephrotoxicity in rats.

Keywords: *nephroprotective effect, Malus domestica, gentamicin.*

1. Introduction

Kidney function is crucial for maintaining our body's overall hemostasis. This essential organ aids in the regulation and synthesis of several key hormones, including the erythropoietin needed for the creation of red blood cells, blood pressure regulation, and the manufacture of hematite, as well as detoxification, acid-base and hydro-mineral balance [1]. Since the kidney has the ability to excrete toxic substances, drug-induced nephrotoxicity is a highly prevalent complication of kidney disorders affecting people all over the world. An aminoglycoside antibiotic called gentamicin, which is frequently used to treat Gram-negative bacterial infections, has been shown to be nephrotoxic in both humans and animals, however prevention methods are still being researched [2].

It is well established that reactive oxidative species (ROS) and the subsequent oxidative stress play an important role in the development of gentamicin-induced nephrotoxicity, which alters the oxidant–antioxidant balance and disrupt the membrane lipid composition through lipid peroxidation [3]. Gentamicin administration is involved in production of hydrogen peroxides, superoxide anions and hydroxyl radicals in the renal cortical mitochondria, concomitant with the reduced efficiency of antioxidant enzymes, indicating the involvement of oxidative stress in tubular, glomerulus and vascular damage [4].

Malus domestica (apple), belonging to the family Rosaceae is one of the most available fruit rich in antioxidant polyphenols including flavonoids, procyanidin, hydroxycinnamic acids and dihydrochalcones which are concentrated high in apple peel than in flesh [5]. Ameliorative effects of the apple peel extract and apple pulp extract have been reported in various studies, such as experimental colitis [6], plasma lipids of atherogenic rats and cholesterol-fed rats [7]. Polyphenolic apple peel extract protected the gastric, intestinal and colonic mucosa from oxidative stress by preventing increased MDA in rats treated with indomethacin (40 mg/kg) [8]. Oral administration of extract showed anti-inflammatory effect on H. pylori associated gastritis and lowering MDA levels [9]. Moreover, in a diabetic pancreas model, Apple Peel Extract showed promising effects in decreasing MDA, NF-κB and inflammatory cytokines [10]. The aim of the present study was to investigate the effect of ethanolic extract of *Malus domestica* peel against gentamicin-induced nephrotoxicity in a rat model.

2. Materials and Methods

Reagents

Gentamicin (ABBOTT Laboratories) was purchased from a pharmacy. Commercial kits for the assay of serum urea, uric acid and creatinine were obtained from ARKRAY Healthcare Pvt Ltd.

All other chemicals and reagents used for phytochemical screening of extract and biochemical assays were purchased from a commercial source at the analytical pure grade.

Preparation of ethanolic extract of *M. domestica*

Fresh peels of *M. domestica* (apple) were collected and made into a coarse powder with the help of an electric grinder. About 300 g of coarse material was mixed with 2100 ml (70%) ethanol using an electric blender and the mixture was shaken in a thermostatic water bath at 40° C for 4 h. The residue was removed by filtration using filter paper (Whattman No. 40) and the filtrate was concentrated by evaporation using rotary evaporator [11]. The brown molten mass was obtained and stored in the refrigerator (8° C) until use. The extract was dissolved in distilled water for oral administration to rats.

Experimental Animals

Wistar rats (200-250 g) of male sex were procured from Sainath Agencies, (CPCSEA Reg. no: 320/CPCSEA) Animals were housed at CPCSEA approved Animal House of, G. Pulla Reddy College of Pharmacy, Hyderabad. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 hrs light and 12 hrs dark cycle) and had free access to commercial pellet diet with water *ad libitum* at 25 ± 2° C with relative humidity at 50 ± 20 %. The study was approved by the Institutional Animal Ethics Committee, G. Pulla Reddy College of Pharmacy (02/29/21/2021/PCL-6). Ethical norms were strictly followed during all the experiments.

Experimental Design

The study was intended for 28 days, using Male Wistar rats weighing 200-250 g. Experimental animals were randomly divided into five groups, each group containing 6 animals as follows,
Normal Control, Disease Control and three Treatment groups with different doses. Rats in the Disease Control, received a single dose of 80 mg/kg of gentamicin, i.p, for 7 days (22^{nd} -28^{th} days), which is responsible for disease induction [12]. Treatment Groups which received *Malus domestica* peel extract (MDPE) with different doses (100 mg/kg, 200 mg/kg, 400 mg/kg., p.o.) respectively for 28 days and at last for 7 days (22^{nd} -28^{th} days), single dose of 80 mg/kg of gentamicin, i.p was administered after 3 h of treatment.

Sample Collection and Biochemical Assays

24 hours after treatment, blood was collected through retro-orbital plexus using ketamine (50 mg/kg, i.p) as anaesthetic. Serum was separated by centrifugation for biochemical estimations. Serum levels of urea, uric acid and creatinine were measured for monitoring the renal function by analyzing the biomarkers using commercially available standard kits (ARKRAY Healthcare Pvt Ltd) in autoanalyzer (star21 plus). Thereafter, the animals were sacrificed by CO2 overdose and the kidneys were isolated carefully and weighed. Kidneys were used for biochemical estimation of oxidative stress parameter i.e., malondialdehyde (MDA) [13] and antioxidant parameters i.e., superoxide dismutase (SOD) [14] and catalase (CAT) [15] and histopathological studies.

Statistical Analysis

The data were analysed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test in Graphpad prism 9.4.0 version and the results were expressed as mean ± SEM. P value < 0.05 was considered statistically significant.

3. Results

Percentage Yield of MDPE

The percentage yield of extract obtained from extraction of fresh peels of *Malus domestica* using ethanol as solvent was found to be 17.7% w/w.

Phytochemical screening of MDPE

Phytochemical screening of MDPE was carried out qualitatively. Various tests were used to identify the presence of alkaloids, carbohydrates, glycosides, proteins, flavonoids, phenolic compounds, saponins, tannins, phytosterols, anthocyanins and coumarins.
The phytochemical investigation of MDPE revealed the presence of carbohydrates, proteins, flavonoids, phenolic compounds, tannins, phytosterols and coumarins.

Effect of MDPE on Gentamicin-Induced Alterations in Kidney Weights

(Table 1) shows the effect of MDPE on kidney weights in various groups. The results clearly indicate that there was a significant increase in kidney weights of disease control group compared to the normal control group and on the other hand, treatment with MDPE (200 mg/kg and 400 mg/kg., p.o.) led to significant decrease in kidney weights compared to disease control.

Table 1. Effect of MDPE on kidney weights

S.NO	GROUPS	KIDNEY WEIGHT (g)
1.	Normal control	0.94 ± 0.020
2.	Disease control	$1.05 \pm 0.006^{\alpha}$
3.	MDPE (100 mg/kg)	1.05 ± 0.016
4.	MDPE (200 mg/kg)	$0.83 \pm 0.025^{****}$
5.	MDPE (400 mg/kg)	$0.86 \pm 0.026^{****}$

Data was analysed using one way ANOVA followed by Tukey's Multiple Comparison Test and expressed in mean ± SEM.
($^{\alpha}$ $P < 0.0001$) Disease control vs normal control, ($^{****}P < 0.0001$) Disease control vs MDPE (200 mg/kg and 400 mg/kg)

Effect of MDPE on Gentamicin-Induced Changes in Serum Biomarkers of Renal function

Serum biomarkers of renal function such as urea, uric acid and creatinine were estimated in this study. Administration of gentamicin (80 mg/kg, i.p.) for 7 days significantly ($P < 0.01$) increased the levels of urea, uric acid and creatinine in disease control compared to normal control. Treatment with MDPE (100 mg/kg., p.o.) significantly ($P< 0.01$) decreased the levels of creatinine, whereas treatment of rats with MDPE (200 mg/kg and 400 mg/kg., p.o.) led to significant decrease of urea, uric acid and creatinine compared to disease control as shown in (Table 2)

Table 2. Effect of MDPE on Serum Biomarkers

S.NO	GROUPS	UREA (mg/dL)	URIC ACID (mg/dL)	CREATININE (mg/dL)
1.	Normal control	15.73 ± 2.328	2.87 ± 0.556	0.95 ± 0.189
2.	Disease control	56.80 ± 10.86 $^{\alpha}$	7.338 ± 0.246 $^{\alpha}$	4.6 ± 0.828 $^{\alpha}$
3.	MDPE (100 mg/kg)	46.06 ± 5.293	5.268 ± 1.305	1.7 ± 0.450 **
4.	MDPE (200 mg/kg)	23.12 ± 1.292 **	3.018 ± 0.632 **	0.85 ± 0.095 ***
5.	MDPE (400 mg/kg)	19.86 ±3.248**	1.643 ± 0.170 ***	0.5 ± 0.129 ****

Data was analysed using one way ANOVA followed by Tukey's Multiple Comparison Test and expressed in mean ± SEM. Comparisons were done between (a) Disease control vs normal control ($^{\alpha}$P < 0.01) and (b) Disease control vs MDPE (100 mg/kg, 200 mg/kg and 400 mg/kg) (**P< 0.01, *** P < 0.001, **** P < 0.0001) were the significance values observed.

Effect of MDPE on Gentamicin-Induced Lipid Peroxidation

MDA levels of lipid peroxidation in kidneys were assessed as a biomarker for oxidative stress. In normal control, the MDA level was found to be 0.972 ± 0.093 µmol/mg. In the Disease control group, gentamicin administration (80 mg/kg i.p.) produced a significant (P < 0.01) increase in the levels of MDA compared to the Normal control group. Treatment with (100 mg/kg, p.o.) of MDPE for 28 days has not significantly decreased gentamicin-induced lipid peroxidation whereas, treatment with MDPE (200 mg/kg and 400 mg/kg, p.o.), shown a significant (P < 0.05 and P < 0.01, respectively) decrease in lipid peroxidation compared to Disease control as shown in (Table 3).

Effect of MDPE on SOD Enzyme Activity

Renal SOD levels were estimated as a biomarker for antioxidant enzymes, in normal control it was found to be 13.38 ± 2.249 U/mg. A significant (P < 0.01) decrease in the levels of SOD was observed in the Disease control group treated with gentamicin (80 mg/kg, i.p.) when compared to a normal control group. Treatment with MDPE (100 mg/kg, p.o.) for 28 days no significant decrease was noted in the levels of SOD compared to disease control. Whereas, treatment of rats with MDPE (200 mg/kg and 400 mg/kg, p.o.) for 28 days a significant (P < 0.05 and P < 0.01, respectively) increase in the levels of SOD was observed compared to Disease control group as shown in (Table 3).

Effect of MDPE on CAT Enzyme Activity

CAT levels of the kidney were estimated as an antioxidant enzyme biomarker and in normal control it was found to be 15.14 ± 1.449 U/mg. Administration of gentamicin (80 mg/kg, i.p.) in Disease control, significantly (P < 0.05) decreased the levels of CAT compared to normal control group. Treatment with MDPE (100 mg/kg) no significant decrease was noted in the levels of CAT compared to disease control and the levels of CAT were significantly (P < 0.01 and P < 0.001) increased in MDPE (200 mg/kg and 400 mg/kg, respectively) groups compared to disease control as shown in (Table 3).

Table 3. Effect of MDPE on Lipid Peroxidation, SOD and CAT

S.NO	GROUPS	MDA (µmol/mg)	SOD (U/mg)	CAT (U/mg)
1.	Normal control	0.972 ± 0.093	13.38 ± 2.249	15.14 ± 1.449
2.	Disease control	6.880 ± 2.204 $^{\alpha}$	2.875 ± 0.826 $^{\alpha}$	9.285 ± 0.735 $^{\alpha}$
3.	MDPE (100 mg/kg)	3.123 ± 0.545	5.125 ± 1.297	9.593 ± 1.301
4.	MDPE (200 mg/kg)	2.225 ± 0.129*	9.500 ± 0.736*	15.28 ± 1.938**
5.	MDPE (400 mg/kg)	1.183 ± 0.248 **	12.75 ± 1.843**	18.67 ± 0.707***

Data was analysed using one way ANOVA followed by Tukey's Multiple Comparison Test and expressed in mean ± SEM. Comparisons were done between (a) Disease control vs normal control, in case of MDA and SOD ($^{\alpha}$ $P < 0.01$), and in the case of CAT ($^{\alpha}$ $P < 0.05$), were the significance values observed and (b) Disease control vs MDPE (100 mg/kg, 200 mg/kg and 400 mg/kg)
($^{*}P< 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) were the significance values observed.

Histopathology

Histological evaluation of kidneys of normal control group illustrated a normal architecture. The morphology of glomerulus was clear, capsular spaces were small, the structure of epithelial cells in proximal convoluted tubules and distal convoluted tubules was normal and devoid of any congestion, necrosis and inflammatory infiltration (Figure 1). In the gentamicin-treated group, the glomerulus was disintegrated, capsular spaces were expanded, degeneration and inflammation of proximal convoluted tubules and distal convoluted tubules. The structure was not clear and dilatation of tubules was noticed (Figure 2).

Kidney tissue examined from MDPE (100 mg/kg., p.o.) treated rats, Cystic dilatation or degeneration was observed in the epithelial cells of proximal convoluted tubules and distal convoluted tubules. The glomerulus morphology was moderately disintegrated, capsular spaces were small (Figure 3). In case of MDPE (200 mg/kg) treated rats depicted, Normal morphology of glomerulus and Mild dilatation and degeneration in the epithelial cells of proximal convoluted tubules and distal convoluted tubules, capsular spaces were also smaller compared to disease control histological alterations (Figure 4). Whereas, in case of MDPE (400 mg/kg) treated group, Normal architectures (intact glomerular basement membrane and tubules) of kidney tissue were observed compared to disease control (Figure 5).

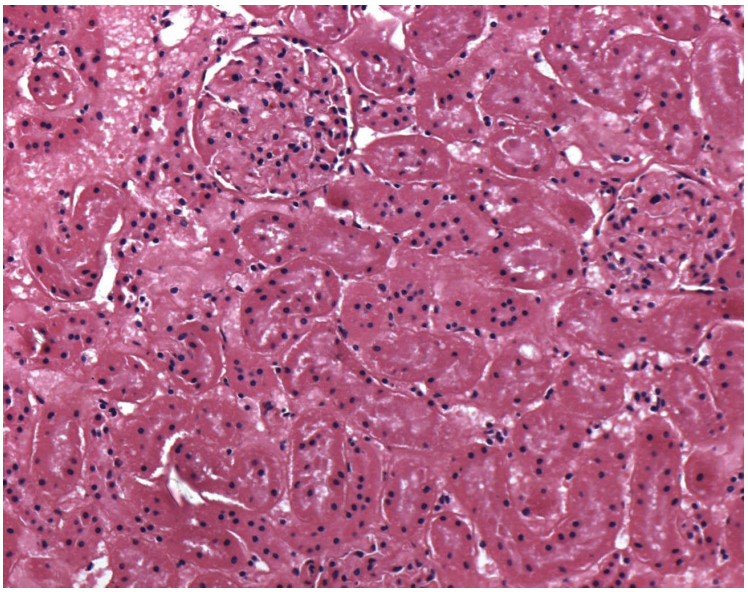

Figure 1: Histology of kidney of Normal control

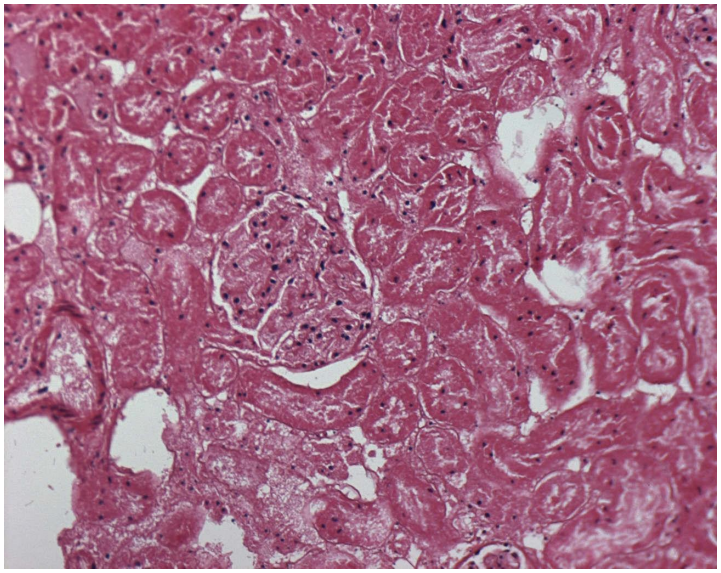

Figure 2: Histology of kidney of Disease control

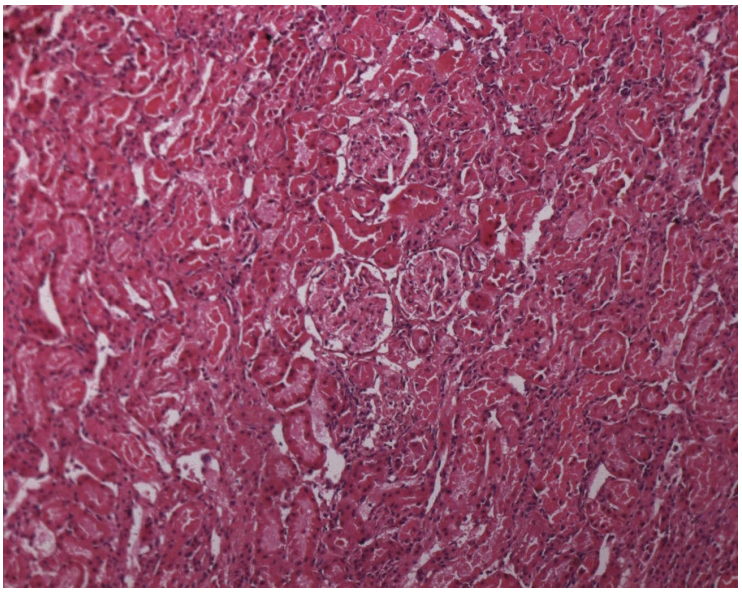

Figure 3: Histology of kidney of MDPE (100 mg/kg)

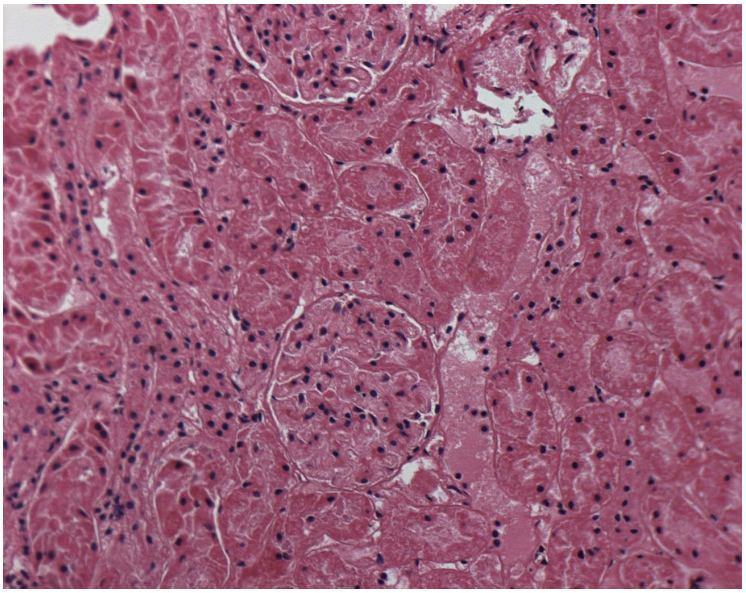

Figure 4: Histology of kidney of MDPE (200 mg/kg)

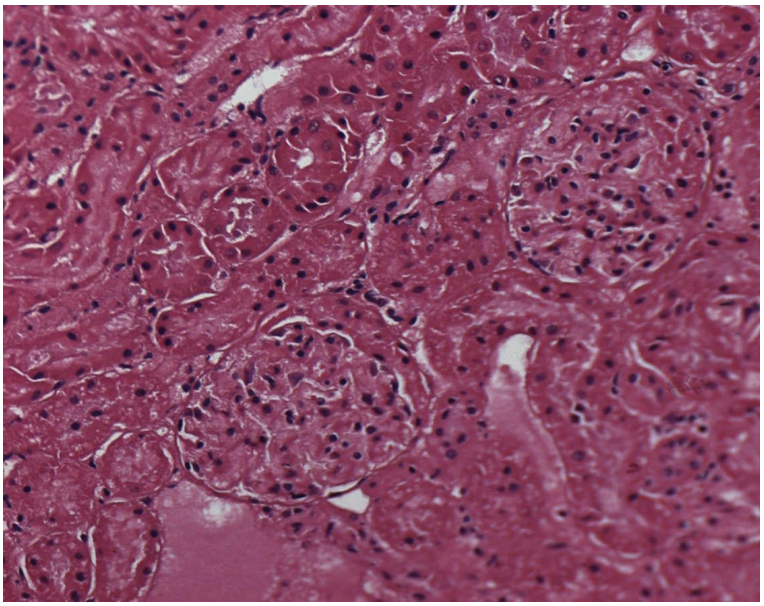

Figure 5: Histology of kidney of MDPE (400 mg/kg)

4. Discussion

Gentamicin-induced nephrotoxicity is characterized functionally by an increase of serum creatinine, blood urea nitrogen, urea, uric acid, NGAL, KIM-1 decrease in GFR [16,17] and morphologically characterized by proximal tubule epithelial desquamation, tubular necrosis, tubular fibrosis, epithelial edema and glomerular hypertrophy [16,18]. It is well established that ROS and the subsequent oxidative stress play an important role in the development of gentamicin-induced nephrotoxicity, which alters the oxidant–antioxidant balance and disrupt the membrane lipid composition through lipid peroxidation and subsequently increase the MDA, a final metabolite product of lipid peroxidation [19,20].

SOD and CAT are the dynamic antioxidant enzymes which convert oxygen molecules into non-toxic products. Several studies reported that the decline in these antioxidant enzymes is mainly due to more ROS and lipid peroxidation. Gentamicin administration is involved in production of hydrogen peroxides, superoxide anions and hydroxyl radicals in the renal cortical mitochondria, concomitant with the reduced efficiency of antioxidant enzymes such as SOD [19,21], catalase [22], indicating the involvement of oxidative stress in nephrotoxicity.

Therefore, application of antioxidant drugs may be beneficial to reverse the renal damage caused by administration of gentamicin. In previous studies, it has been reported that *Malus domestica* contains significant amounts of polyphenols [23] with effective antioxidant activity [11], thus in this research ethanolic extract of *Malus domestica* peels has been selected for assessing its potential protective activity on gentamicin-induced nephrotoxicity in rats.

Serum urea and creatinine are the major indicators or markers of renal function [24]. The elevation in creatinine levels in serum may be due to the abnormality of glomerular filtration process in the kidney. In the present study, gentamicin administration (80 mg/kg i.p.) to wistar rats resulted in nephrotoxicity [12], with a significant increase in serum urea, uric acid and creatinine observed in Disease control group compared to normal control group. As evident from the results, the pre-treatment of rats with MDPE significantly attenuated the elevated levels of urea, uric acid and creatinine induced by gentamicin in MDPE (200 mg/kg and 400 mg/kg p.o.) treatment groups. Protective effects of antioxidants over Gentamicin-induced altered renal biochemical indices have been reported.

In this study, the significant increase in renal tissue MDA levels in gentamicin-treated rats was consistent with previous reports associated with overproduction of free radicals in

gentamicin-induced nephrotoxicity. Gentamicin-induced increment in MDA levels was significantly prevented by MDPE treatment in the present study. Therefore, the significantly lower levels of MDA in the kidney tissues of treated groups as compared with the Disease control group indicates attenuation of lipid peroxidation.

In accordance with the previous findings, results showed significant decrease in the levels of SOD and CAT in disease control group administered with gentamicin. The present finding indicated that treatment with MDPE for 28 days restored the levels of SOD and CAT after gentamicin administration in MDPE (200 mg/kg and 400 mg/kg) groups, whereas in MDPE (100 mg/kg) group no significant change was observed, showing an effect in a dose dependent manner.

The results of serum markers and antioxidant parameters of nephrotoxicity were correlated with histopathological examination. The gentamicin treated group exhibited disintegration of glomerulus, interstitial inflammation, degeneration and dilatation of tubules. The MDPE (100 mg/kg) group showed no difference in histological changes when compared to disease control group, whereas in MDPE (200 mg/kg) group mild degeneration of tubules with normal morphology of glomerulus and no MDPE (400 mg/kg) group morphological changes of glomerulus, smaller capsular spaces and no degeneration of tubules was observed compared to disease control group.

The nephroprotective effect of MDPE was evidenced by improvement in the structural morphology of renal profiles and suppression of oxidative stress. Treatment with MDPE significantly augmented the majority of the deleterious effects produced by gentamicin. Thus, oral administration of MDPE revealed significant potential ameliorative effects against toxic changes in serum markers, antioxidants and histopathology, brought by gentamicin by its renal accumulation and damage by reactive oxygen species.

5. Conclusion

Gentamicin-induced nephrotoxicity was contributed due to oxidative stress, leading to increasing the levels of renal function biomarkers (urea, uric acid and creatinine), lipid peroxidation and reduced antioxidant enzymes. Pretreatment of animals with MDPE dose-dependently and significantly reversed the changes of serum biomarkers, MDA, SOD and CAT. Thereby, from all the findings of this study it was concluded that ethanolic extract of *Malus domestica* peel has a potential protective effect against gentamicin-induced nephrotoxicity.

6. Acknowledgement

The authors would like to acknowledge G. Pulla Reddy College of Pharmacy, Hyderabad, India, for funding and providing necessary facilities to carry out the study.

References

1. Jose, Svenia P., S. Asha, I. M. Krishnakumar, M. Ratheesh, Savitha Santhosh, S. Sandya, Girish Kumar, and C. Pramod. "Nephro-protective effect of a novel formulation of unopened coconut inflorescence sap powder on gentamicin induced renal damage by modulating oxidative stress and inflammatory markers." *Biomedicine & Pharmacotherapy* 85 (2017): 128-135.
2. Ehsani, Vahid, Morteza Amirteimoury, Zahra Taghipour, Ali Shamsizadeh, Gholamreza Bazmandegan, Amir Rahnama, Fatemeh Khajehasani, and Iman Fatemi. "Protective effect of hydroalcoholic extract of Pistacia vera against gentamicin-induced nephrotoxicity in rats." *Renal failure* 39, no. 1 (2017): 519-525.
3. Beshay, Olivia N., Mohamed G. Ewees, Mohamed S. Abdel-Bakky, Sara Mohamed Naguib Abdel Hafez, Amany B. Abdelrehim, and Asmaa MA Bayoumi. "Resveratrol reduces gentamicin-induced EMT in the kidney via inhibition of reactive oxygen species and involving TGF-β/Smad pathway." *Life Sciences* 258 (2020): 118178.
4. Lopez-Novoa, J.M., Quiros, Y., Vicente, L., Morales, A.I. and Lopez-Hernandez, F.J., 2011. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. *Kidney international*, *79*(1), pp.33-45.
5. Boyer, Jeanelle, and Rui Hai Liu. "Apple phytochemicals and their health benefits." *Nutrition journal* 3, no. 1 (2004): 1-15.

6. Pastrelo, Maurício Mercaldi, Carla Caroline Dias Ribeiro, Joselmo Willamys Duarte, Andréa Pitelli Bioago Gollücke, Ricardo Artigiani-Neto, Daniel Araki Ribeiro, Sender Jankiel Miszputen, Celina Tizuko Fujiyama Oshima, and Ana Paula Ribeiro Paiotti. "Effect of concentrated apple extract on experimental colitis induced by acetic acid." *International journal of molecular and cellular medicine* 6, no. 1 (2017): 38.
7. Leontowicz, H. A. N. N. A., M. A. R. I. A. Leontowicz, S. H. E. L. A. Gorinstein, O. L. G. A. Martin-Belloso, and S. I. M. O. N. Trakhtenberg. "Apple peels and pulp as a source of bioactive compounds and their influence digestibility and lipid profile in normal and atherogenic rats-in English." *Medycyna weterynaryjna* 63 (2007): 1434.
8. Carrasco-Pozo, Catalina, Hernán Speisky, Oscar Brunser, Edgar Pastene, and Martin Gotteland. "Apple peel polyphenols protect against gastrointestinal mucosa alterations induced by indomethacin in rats." *Journal of Agricultural and Food Chemistry* 59, no. 12 (2011): 6459-6466.
9. Pastene, Edgar, Hernan Speisky, Apolinaria García, Jessica Moreno, Miriam Troncoso, and Guillermo Figueroa. "In vitro and in vivo effects of apple peel polyphenols against Helicobacter pylori." *Journal of agricultural and food chemistry* 58, no. 12 (2010): 7172-7179.
10. Fathy, Samah M., and Ehab A. Drees. "Protective effects of Egyptian cloudy apple juice and apple peel extract on lipid peroxidation, antioxidant enzymes and inflammatory status in diabetic rat pancreas." *BMC complementary and alternative medicine* 16, no. 1 (2015): 1-14.
11. Issa, Najlaa K., R. Abdul Jabar, Y. Hammo, and I. Kamal. "Antioxidant activity of apple peels bioactive molecules extractives." *Science and technology* 6, no. 3 (2016): 76-88.
12. Erseçkin, Vasfiye, Handan Mert, Kıvanç İrak, Serkan Yildirim, and Nihat Mert. "Nephroprotective Effect of Ferulic Acid on Gentamicin-Induced Nephrotoxicity in Female Rats." Drug and Chemical Toxicology 45, no. 2 (2020): 663–69.
13. Buege, John A., and Steven D. Aust. "[30] Microsomal lipid peroxidation." *Methods in enzymology* 52 (1978): 302-310.
14. Marklund, Stefan, and Gudrun Marklund. "Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase." *European journal of biochemistry* 47, no. 3 (1974): 469-474.
15. Hadwan, Mahmoud Hussein, and Hussein Najm Abed. "Data supporting the spectrophotometric method for the estimation of catalase activity." *Data in brief* 6 (2016): 194-199.
16. Hassanein, Emad H., Fares E. Ali, Magy R. Kozman, and Omnia A. Abd El-Ghafar. "Umbelliferone Attenuates Gentamicin-Induced Renal Toxicity by Suppression of TLR-4/NF-KB-P65/NLRP-3 and JAK1/STAT-3 Signaling Pathways." Environmental Science and Pollution Research 28, no. 9 (2020): 11558–71.
17. Han, Chunyang, Taotao Sun, Yawei Liu, Guangtai Fan, Wanjun Zhang, and Cuiyan Liu. "Protective effect of Polygonatum sibiricum polysaccharides on gentamicin-induced acute kidney injury in rats via inhibiting p38 MAPK/ATF2 pathway." *International journal of biological macromolecules* 151 (2020): 595-601.
18. Saleem, Mohammad, Fatima Javed, Muhammad Asif, Muhammad Kashif Baig, and Mehwish Arif. "HPLC analysis and in vivo renoprotective evaluation of hydroalcoholic extract of Cucumis melo seeds in gentamicin-induced renal damage." *Medicina* 55, no. 4 (2019): 107.
19. Famurewa, A.C., Maduagwuna, E.K., Folawiyo, A.M., Besong, E.E., Eteudo, A.N., Famurewa, O.A. and Ejezie, F.E. (2019). Antioxidant, anti‐inflammatory, and antiapoptotic effects of virgin coconut oil against antibiotic drug gentamicin‐induced nephrotoxicity via the suppression of oxidative stress and modulation of iNOS/NF‐κB/caspase‐3 signaling pathway in Wistar rats. Journal of Food Biochemistry, 44(1).
20. Adil, Mohammad, Amit D. Kandhare, Gautami Dalvi, Pinaki Ghosh, Shivakumar Venkata, Kiran S. Raygude, and Subhash L. Bodhankar. "Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction." *Renal failure* 38, no. 6 (2016): 996-1006.
21. Cao, Liying, Dongyun Zhi, Jing Han, Sushil Kumar Sah, and Yunhui Xie. "Combinational effect of curcumin and metformin against gentamicin‐induced nephrotoxicity: Involvement of antioxidative, anti‐inflammatory and antiapoptotic pathway." *Journal of food biochemistry* 43, no. 7 (2019): e12836.
22. Edeogu, C. O., Michael E. Kalu, Ademola C. Famurewa, Nnaemeka T. Asogwa, Gertrude N. Onyeji, and Kelechi O. Ikpemo. "Nephroprotective effect of Moringa oleifera seed oil on gentamicin-induced nephrotoxicity in rats: biochemical evaluation of antioxidant, anti-inflammatory, and antiapoptotic pathways." *Journal of the American college of nutrition* 39, no. 4 (2020): 307-315.
23. Nkuimi Wandjou, Joice G., Stefania Sut, Claudia Giuliani, Gelsomina Fico, Fabrizio Papa, Stefano Ferraro, Giovanni Caprioli, Filippo Maggi, and Stefano Dall'Acqua. "Characterization of nutrients, polyphenols and volatile components of the ancient apple cultivar 'Mela Rosa Dei Monti Sibillini'from Marche region, central Italy." *International journal of food sciences and nutrition* 70, no. 7 (2019): 796-812.
24. Tavafi, Majid, and Hasan Ahmadvand. "Effect of rosmarinic acid on inhibition of gentamicin induced nephrotoxicity in rats." *Tissue and Cell* 43, no. 6 (2011): 392-397.