International Journal of Pharmacology

ISSN 1811-7775

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.994.1003

Research Article
Renoprotective and *in silico* Modeling Studies of Febuxostat in Gentamicin Induced Nephrotoxic Rats

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Abstract

Background and Objective: Febuxostat is used for the treatment of hyperuricemia that improves renal function in CKD patients, but its renal protective effect has not been fully studied. This study has been undertaken to evaluate the potential modulatory effects of febuxostat on renal dysfunction and kidney injury in an experimental model of gentamicin-induced nephrotoxicity in rats. **Materials and Methods:** These effects were assessed in terms of analysing biochemical (creatinine, urea, blood urea nitrogen, uric acid, serum electrolytes, alanine aminotransferase, aspartate transaminase and alkaline phosphatase, among others) parameters, total histological kidney score, as well as the histological assay of kidney and liver tissues. **Results:** As compared to gentamicin control (100 mg kg^{-1}) groups, the febuxostat (10 mg kg^{-1}, i.p.) pre/post-treatment was significantly preventive in terms of biochemical parameters and the total histological score of kidney tissue. Either pre/post-treatment markedly reduced the changes of glomerular enlargement, degeneration tubular hyaline casts and tubular degeneration, while the liver showed no remarkable changes in hepatocytes or liver architecture. **Conclusion:** This observation may have a great impact on the use of aminoglycosides, whereas febuxostat may have a key potential to prevent the associated renal toxicity induced by the administration of gentamicin.

Key words: Febuxostat, gentamicin, nephrotoxicity, histopathology *in silico*, ADME/T, renoprotection, pretreatment

Citation: Alqahtani, A., K. Chidambaram, K. Asseri, K. Venkatesan and A.A. Qureshi *et al*., 2022. Renoprotective and *in silico* modeling studies of febuxostat in gentamicin induced nephrotoxic rats. Int. J. Pharmacol., 18: 994.1003.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gentamicin, which is useful in the management of infections caused by gram-negative bacteria, belongs to the aminoglycosides group (AG) of antibiotics[1]. The AG is a highly efficient antibiotic employed for the management of complicated infections[2]. However, the use of gentamicin is associated with severe complications, such as ototoxicity (ear poisoning), nephrotoxicity (renal toxicity), neuromuscular blockade and allergic reactions[3]. A single dose of gentamicin administration can lead to acute kidney damage[4,5]. The nephrotoxicity induced by gentamicin is associated with increases in the serum creatinine and blood urea levels and the induction of acute tubular necrosis[6-8]. Gentamicin-induced nephrotoxicity is identified by tubular necrosis without changing the morphology of glomerular structures[9], which may be caused by the generation of reactive oxygen species (ROS)[10]. The ROS overproduction damages macromolecules and causes cellular injury and necrosis through various mechanisms, such as lipid peroxidation, protein denaturation and DNA damage[11]. Hepatic cells were also affected by gentamicin, which induced structural and functional changes. NF-B and its downstream pro-inflammatory cytokines have been implicated in the aetiology of GNT-induced hepatotoxicity[12]. Given that inflammation plays a significant part in the development of kidney injury, in many preclinical and clinical trials, drugs with nephroprotective and hepatoprotective effects along with anti-inflammatory and antioxidant properties were examined[13-15].

Febuxostat is a non-purine selective potent inhibitor of xanthine oxidase[16,17]. Febuxostat is used for the treatment of hyperuricemia and gout, while it has demonstrated antioxidant, cytoprotection and anti-inflammatory effects[18-20]. It is the most used drug in clinics for the treatment of hyperuricemia, can lower the serum uric acid levels through targeting xanthine oxidase[21,22]. Experimental research has shown that febuxostat has nephroprotective effects and it has recently been shown to enhance renal function in patients with CKD stage 3[23]. In hyperuricemic individuals with CKD, febuxostat appears to reduce serum uric acid levels and prevent the progression of renal impairment more effectively than allopurinol in several experimental and randomized controlled trials[24-27]. Several recent experiments have revealed that febuxostat protects rats from toxin-induced cardiac and renal damage[28,29].

Febuxostat has been shown to have beneficial effects in drug-induced renal injury, suggesting that it suppresses apoptosis and reduces oxidative stress[28,30-33]. Febuxostat has superior renal safety to allopurinol, but data on its hepatic safety are limited. However, its reno-protective and hepatoprotective properties on gentamicin-induced kidney injury have yet to be fully studied. Febuxostat at a dose of 40 mg/day (3-6 months) has a positive effect in patients with chronic kidney disease, which is reported in recent clinical studies[34,35]. Hence, the present study was undertaken to determine whether pre/post-treatment with febuxostat can alleviate the major traits of gentamicin-induced renal and hepatic hemodynamic alterations. Additionally, drug-like and toxicological properties (ADME/T) were assessed to evaluate the drug-likeness properties.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Saudi Arabia, from August to December, 2020.

Chemicals: Febuxostat and gentamicin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in the present study are of analytical grade.

Animals: Thirty male Wistar Albino rats of 8-10 weeks old weighing 200-250 g were procured from Central Animal House Facility (CAHF), King Khalid University, Abha, Saudi Arabia. The experimental rats were kept in Central Animal House Facility under natural lighting conditions (12 hrs light and 12 hrs dark cycle) with a temperature of 22-25°C and relative humidity of approximately 50%. The experimental animals had free access to a standard rodent diet (Bio-Serv, Canada) and water *ad libitum*. The experimental design employed in the present study was approved by the Institutional Ethics Committee (IEC), King Khalid University (ECM#2020-3202). All animal experiments were carried out according to the protocol authorized by the IEC and the NIH-recommended International Guidelines for Animal Welfare.

Gentamicin-induced nephrotoxicity: Totally 30 rats were divided into five experimental groups, each comprising six animals. The first group (Vehicle Control) served as normal control was maintained on standard food and water orally. The second group (Gentamicin Control) was intraperitoneally (i.p.) administered with gentamicin for 8 days, at a dose of 100 mg kg^{-1}/day[36]. The third group (Febuxostat *per se*) was administered only with febuxostat at a dose of 10 mg kg^{-1} in

CMC (0.5%) orally for 8 days[27,37,38]. The fourth group (Febuxostat Pre-treated) was administered with gentamicin (100 mg kg^{-1}/day, i.p., 8 days) and febuxostat treatment was given orally for 8 days from a day before the administration of gentamicin. The fifth group (Febuxostat Post-treated) was administered with gentamicin (100 mg kg^{-1}/day, i.p.) for 8 days and later treated with febuxostat for the next 6 days after the completion of 8 days protocol of gentamicin (100 mg kg^{-1}/day, i.p.) administration.

Blood sampling and biochemical parameters: Twenty-four hours after the last treatment of each group, blood samples were collected by cardiac puncture under light isoflurane anaesthesia. The blood samples were centrifuged for 15 min at 1000×g after 2 hrs at room temperature. The serum samples were stored at -20°C for 48 hrs until further use. Serum biochemical markers of renal dysfunction and hepatic injury were estimated using commercially available kits according to the manufacturer's protocol (Alpha Laboratories, United Kingdom) using a standard clinical automated analyzer. The biochemical markers estimated in the present study include creatinine, urea, Blood Urea Nitrogen (BUN), uric acid, serum electrolyte (sodium, potassium and calcium) levels, Alanine Aminotransferase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), albumin level and total protein levels. The rats were sacrificed and their kidneys and the liver were isolated for histopathological examination.

Histopathology: The kidney and liver tissues were fixed in 10% buffered formalin solution for 2 days and then dehydration was done using ascending alcohol concentrations (70, 90, 95 and 100%) for 5 min. Subsequently, tissues were cleared in xylene and embedded in paraffin wax. Sections for staining were cut at the thickness of a five-micrometre and were stained using Hematoxylin and Eosin staining. Sections were evaluated by a pathologist blinded to the groups of experimental rats and were examined under a (Pennsylvania, USA) Liver and kidney changes were scored as (0) for minimal or no change, (1) for mild injury characterized by vacuolation in the cytoplasm but no necrosis, (2) for moderate injury showing cytoplasmic vacuolation, eosinophilia and stromal changes in the form of sinusoidal swelling and congestion and (3) for severe injury showing coagulative necrosis and haemorrhage. Renal changes were scored as 0, no or minimal change, 1: Mild, 2: Moderate and 3: Severe for the presence and severity of the following changes: Degeneration of Bowman's space and glomeruli, degeneration of renal tubules and presence of casts, vascular congestion and interstitial edema[39].

***In silico* ADME/T and analysis of toxicological properties:** The drug-likeness properties of febuxostat were evaluated using the Lipinski rule of five. Moreover, the pharmacokinetics properties were also studied. The Lipinski rule of five parameters was checked with the SwissADME http://www.swissadme.ch/index.php#)[40]. The rules are Molecular weight (<500 g mol^{-1}), Hydrogen bond acceptor (<10), Hydrogen bond donor (<5), Log P, Lipophilicity ($\leq$5), several rotatable bonds ($\leq$10) and topological polar surface area ($\leq$140 Å2). Also, the toxicological properties of the febuxostat were assessed using the online tool admetSAR (http://lmmd.ecust.edu.cn/admetsar1/predict/), whereas, AMES toxicity, carcinogenicity, acute oral toxicity and rat acute toxicity were studied[41].

Statistical analysis: Results were expressed as Mean±SEM, $p<0.05$ (*a), $p<0.01$ (**b) and $p<0.001$ (***c) were considered as statistically significant compared with the vehicle control and gentamicin-administered group using one-way analysis of variance (ANOVA) followed by Dunnett's test. The 'p' values were two-tailed.

RESULTS

Effect of febuxostat on biochemical parameters of renal function: Table 1 presented that, the treatment group's creatinine levels were significantly lower, while the groups were compared with the gentamicin control group. A non-significant urea level was observed for all the groups except the febuxostat group (FBX-Per *se*, $p<0.01$). The FBX-Per *se* and FBX-PreT demonstrated a significant ($p<0.05$) reduction in the urea-nitrogen level compared with the vehicle control and gentamicin control groups, respectively. No significant reduction in serum uric acid was observed in FBX-Per *se* and FBX-PreT in comparison with the gentamicin control group.

Effect of febuxostat on serum electrolytes level: The results of the serum electrolytes are presented in Table 2. The gentamicin-induced groups exhibited a significant alteration in the sodium, potassium and calcium levels. The febuxostat administration showed a notable change in all the electrolytes levels when compared with the gentamicin control group.

Table 1: Pre- and post-treatment effects of febuxostat on biochemically analysed renal function parameters in gentamicin-administered nephrotoxic rats.

Groups	Creatinine (mg dL^{-1})	Urea (mg dL^{-1})	BUN (mg dL^{-1})	Uric acid (mg dL^{-1})
Vehicle control	0.47±0.0890.10^{c}	88.60±7.85	41.36±3.67	1.40±0.41
Gentamicin control	1.16±0.108***^{c}	95.60±10.98	44.62±5.14	2.39±0.25
FBX-*per se*	0.42±0.0210.10^{c}	43.83±1.47**^{b}	24.50±0.71*^{a}	1.84±0.255
FBX-preT	0.51±0.0700.10^{c}	92.00±13.64	26.26±7.14*^{a}	1.77±0.47
FBX-postT	0.61±0.0200.10^{c}	90.20±9.22	42.12±4.31	1.79±0.46

Results were shown as mean ± SEM (six rats in each group), $p<0.05^{(*a)}$, $p<0.01^{(**b)}$ and $p<0.001^{(***c)}$ were considered as statistically significant

Table 2: Pre- and post-treatment effects of febuxostat on serum electrolyte levels in gentamicin-administered nephrotoxic rats

Groups	Sodium (mmol L^{-1})	Potassium (mmol L^{-1})	Calcium (mg dL^{-1})
Vehicle control	148.46±1.21^{c}	4.89±0.25^{b}	7.20±0.50^{c}
Gentamicin control	164.96±2.34^{c}	6.70±0.19^{b}	10.70±0.53^{c}
FBX-*per se*	151.46±1.82^{c}	6.61±0.23^{b}	8.16±0.20^{c}
FBX-preT	153.6±1.12^{c}	5.10±0.37^{b}	8.35±0.29^{c}
FBX-postT	155.94±1.73^{b}	5.60±0.48^{a}	10.20±0.28^{c}

Results were shown as Mean±SEM (six rats in each group), $p<0.05^{(a)}$, $p<0.01^{(b)}$ and $p<0.001^{(c)}$ were considered as statistically significant

Table 3: Provided here the histopathologic scores depicting the kidney lesions among different experimental groups

Lesion	Control (A)	Gentamicin (B)	Febuxostat only (C)	Febuxostat pre-treatment (D)	Febuxostat post-treatment (E)
Glomerular enlargement	0	2.32	0	1.87	1.27
Glomerular degeneration	0	2.24	0	1.56	1.93
Tubular degeneration	0	2.73	0	1.43	1.62
Hyaline casts in tubules	0	2.91	0	1.51	1.32

(0) For minimal or no change, (1) Mild injury characterized by vacuolation in the cytoplasm but no necrosis, (2) Moderate injury showing cytoplasmic vacuolation, eosinophilia and stromal changes in the form of sinusoidal swelling and congestion and (3) Severe injury showing coagulative necrosis and haemorrhage

Table 4: ADME/T properties of febuxostat

Physicochemical properties	
Formula	$C_{16}H_{16}N_2O_3S$
Molecular weight	316.37 g mol^{-1}
Number of H-bond acceptors	5
Number of H-bond donors	1
MLOGP	1.46
Number of rotatable bonds	5
Topological polar surface area	111.45 $Å^2$
Pharmacokinetics	
GI absorption	High
BBB permeant	No
Toxicological properties	
AMES toxicity	Non-AMES toxic
Carcinogens	Non-carcinogens
Acute oral toxicity	III
Rat acute toxicity	2.3811

Molecular weight (<500 g moL^{-1}), hydrogen bond acceptor (<10), hydrogen bond donor (<5), Log P, lipophilicity (≤5), number of rotatable bond (≤10), topological polar surface area (≤140 $Å^2$) and category-III means (500 mg kg^{-1}>LD50 <5000 mg kg^{-1})

Effect of febuxostat on liver function parameters and serum protein levels: The effect of febuxostat on liver function parameters and serum protein levels in gentamicin-induced rats is shown in Fig. 1-2, respectively. The ALP levels were noted to be significantly decreased in the febuxostat treated groups (Groups 4 and 5) when compared with the gentamicin-induced group. At the same time, the FBX-PreT (Group 4) showed a significant ($p<0.01$) reduction in the AST level compared with the gentamicin group. On the other hand, an unchanged ALT level was observed for the febuxostat treated groups (Groups 3, 4 and 5). In terms of serum protein parameters, the experimental group had significantly higher total protein levels ($p<0.05$, Fig. 2) than the control group. A non-significant serum albumin level was noted in febuxostat treated groups (Groups 4 and 5).

Histopathological examination: Hematoxylin and Eosin-stained sections from control rats' kidney tissue revealed normal renal architecture, as well as the pyramidal structure of the renal medulla and cortex (Fig. 3a), compared to the gentamicin control's kidney, showed a marked glomerular and tubular injury with gentamicin (Fig. 3b) and febuxostat only group (Fig. 3c). The changes were in the form of glomerular enlargement and degeneration, tubular hyaline casts (indicating severe proteinuria) and tubular degeneration. Either pre-treatment (Fig. 3d) or post-treatment (Fig. 3e) with febuxostat markedly reduced the changes mentioned above (Table 3). Hematoxylin and Eosin-stained sections from the liver showed no remarkable changes in hepatocytes or liver architecture with gentamicin, febuxostat, pre-treatment or post-treatment (Fig. 4a-e).

Drug-likeness properties of febuxostat: Febuxostat follows the Lipinski rule of five, while it also has high GI absorption. Also, it will not cross the blood-brain barrier. The result of the ADME/T analysis has been presented in Table 4. Moreover, the admetSAR study showed that febuxostat was found to be non-AMES, non-carcinogenic and have weak rat toxicity properties.

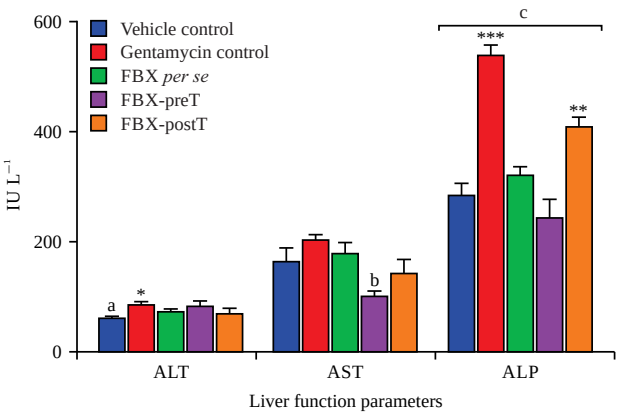

Fig. 1: Pre- and post-treatment effects of febuxostat on liver function parameters in gentamicin-administered nephrotoxic rats

Results were shown as Mean±SEM (six rats in each group), $p<0.05^{(*a)}$, $p<0.01^{(**b)}$ and $p<0.001^{(***c)}$ were considered as statistically significant as compared to the gentamicin group

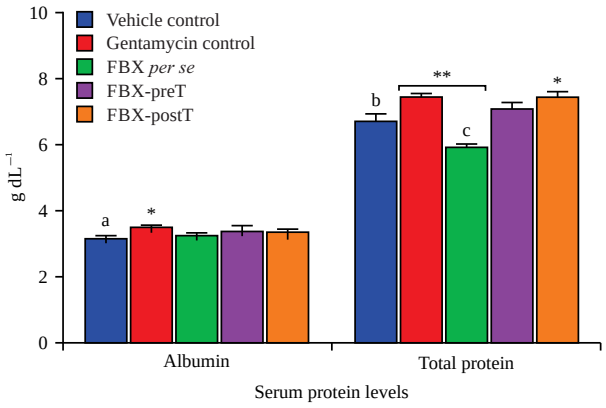

Fig. 2: Pre- and post-treatment effects of febuxostat on serum protein levels in gentamicin-administered nephrotoxic rats

Results were shown as Mean±SEM (six rats in each group), $p<0.05^{(*a)}$ and $p<0.01^{(**b)}$ were considered as statistically significant as compared to the gentamicin group

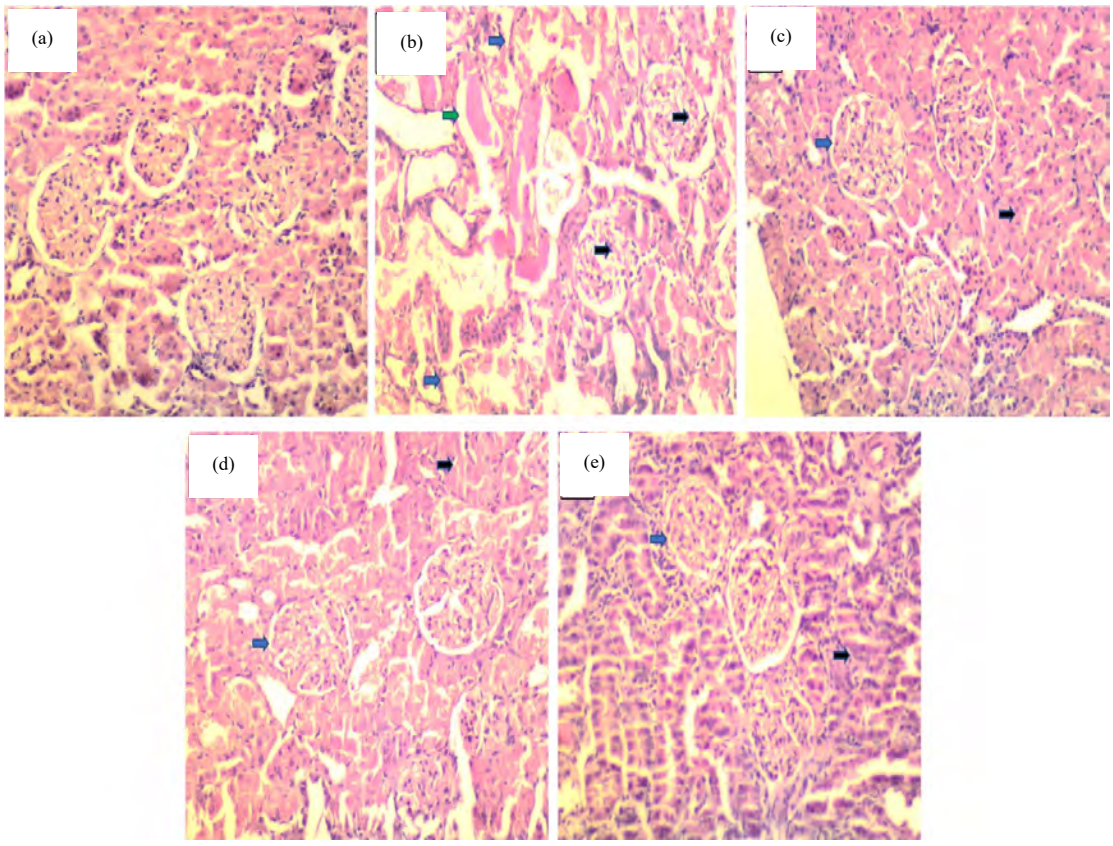

Fig. 3(a-e): Histological presentation of kidney tissues, (a) Control group showing normal renal architecture with numerous healthy glomeruli and tubules, (b) Gentamicin only group showing a marked impairment of the renal architecture with severe glomerular (blue arrows) and tubular degeneration (black arrows) and hyaline casts in the tubular lumens (green arrows), (c) Febuxostat only group showing no significant changes in renal architecture, (d) Febuxostat pre-treatment and Febuxostat post-treatment (e) Showing a marked reduction of gentamicin-induced glomerular injury (blue arrows) and tubular degeneration (black arrows)

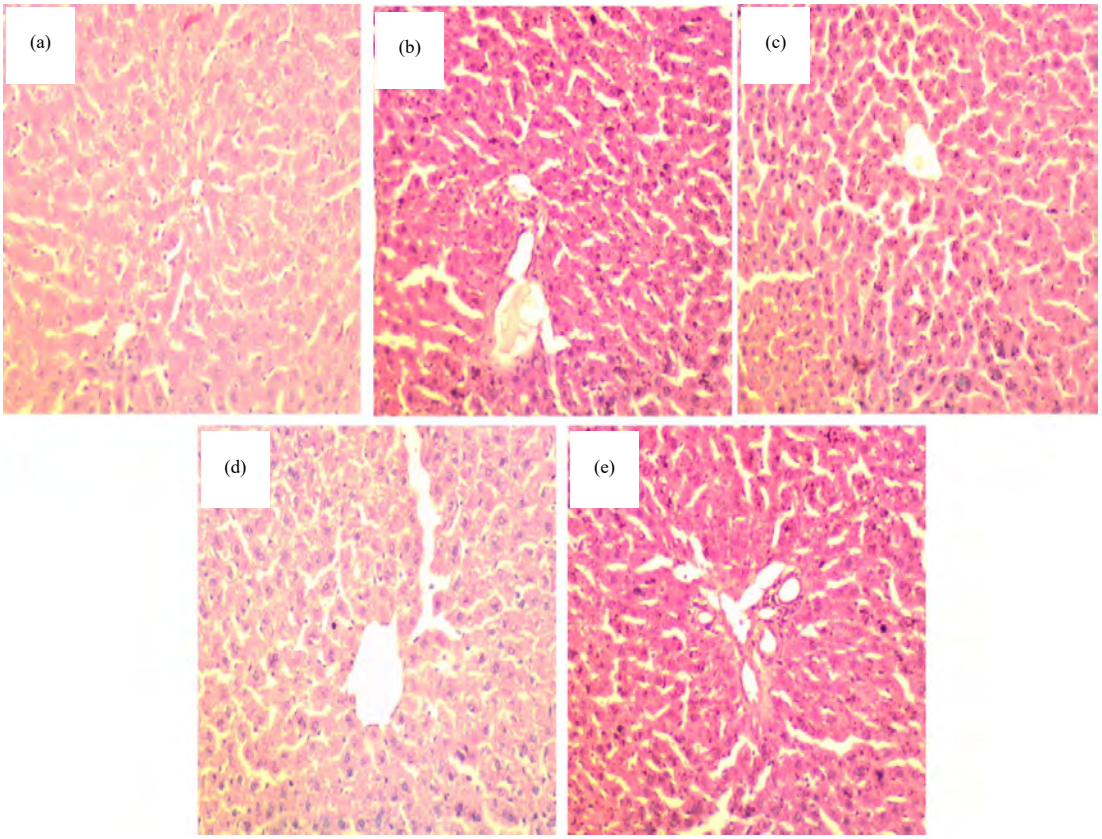

Fig. 4(a-e): Histological presentation of liver tissues, showing no significant changes in the liver between (a) Control, (b) Gentamicin, (c) Febuxostat only, (d) Febuxostat pre-treatment and (e) Febuxostat post-treatment groups

DISCUSSION

The present study revealed that febuxostat has renoprotective properties against gentamicin-induced nephrotoxicity *in vivo*. Drug-likeness properties (ADME/T) of febuxostat were also evaluated using *in silico* methods. The results revealed that pre-and post-treatment treatments of febuxostat demonstrated marked protective effects against gentamicin-induced nephrotoxicity. Proximal tubular epithelia are polarized cell that consists primarily of a basolateral and apical membrane. It has been documented that certain foreign particles are specifically taken up by these epithelial cells through selective carriers and exported via other receptors[42]. The uptake of these xenobiotics on the proximal epithelium cells leading to acute renal failure (ARF) is a common problem. Previously it has been reported that xanthine oxidase (XO) inhibitors improved the renal outcome in the case of the hypertensive patients[43]. Allopurinol, one type of XO inhibitor, competitively inhibits the XO and blocks the formation of uric acid[44]. A study demonstrated that allopurinol reduces the risk of ARF caused by snake venom[45]. However, reversible clinical hepatotoxicity along with ARF may also occur after the allopurinol therapy, which is potentially regarded as an extreme side effect[46].

However, Hu and Brown demonstrated that febuxostat, another type of XO inhibitor, was more effective than allopurinol for the treatment of hyperuricemia and gout[47]. In addition, it has been already reported that febuxostat inhibits XO in both oxidized and reduced forms and has less effect on other purine and pyrimidine enzymes[48]. Moreover, the anti-inflammatory and antioxidant effect of febuxostat is more than that of allopurinol[49,50]. Recently, Omizo *et al.*[51] reported the cardio-renal effects of XO inhibition in rodent Chronic Kidney Disease (CKD) model with hyperuricemia[51]. In the same study, they revealed that febuxostat alleviated arteriolar, glomerular and tubulointerstitial injury in an animal model. Moreover, a meta-analysis by Lin *et al.*[52] depicted the reno-protective effect of febuxostat in CKD patients[52]. Additionally, it has been reported that febuxostat decreases the progression of renal interstitial injury by reducing oxidative stress[53].

Aminoglycosides have long been known for their adverse ability to induce nephrotoxicity. Gentamicin, an aminoglycoside, disrupts renal processes through lysosomal phospholipidosis[6]. Gentamicin-induced nephrotoxicity is functionally characterized by changes in several biochemical parameters, including the higher level of serum creatinine and Blood Urea Nitrogen (BUN) and a decrease in the Glomerular Filtration Rate (GFR)[54].

In the present study, we investigated the potential pre- and post-treatment effects of febuxostat in gentamicin-induced acute nephrotoxicity model in rats. The result of the current study regarding the hepatic biomarkers indicated that treatment with gentamicin causes hepatotoxicity with a significant increase in serum ALT and ALP levels. Importantly, the transaminase and ALP level in serum is considered crucial biomarkers for the normal functioning of the liver. This is due to the cytoplasmic nature and permeability of hepatocyte membranes[55]. In this study, treatment with febuxostat reduced the elevation of hepatic biomarkers. Further, gentamicin caused elevation in serum albumin level as well as total protein level.

Previously, it has been reported that the amount of serum creatinine is relatively higher in patients with renal disorder[56]. Importantly, serum creatinine level is much higher in the late stage of renal failure and hence, it is considered a crucial tool for assessing renal function. In the present experimental study, we assessed the level of serum creatinine after pre- and post-treatment with febuxostat in gentamicin administered rats. The analysis revealed that the amount of serum creatinine was significantly higher when the animals receive gentamicin alone. However, the serum creatinine level was noted to be significantly decreased after the treatment with febuxostat in gentamicin-administered rats. The amount of serum creatinine was noted to be decreased in both the pre- and post-treatment with febuxostat. Additionally, BUN that is related to measuring urea in the kidney and liver is known as an indirect method, although BUN is functionally related to the renal excretory function[57]. Like serum creatinine, gentamicin also increased the BUN in model animals that depicts the functional disorder of the kidney. But the administration of febuxostat gradually reduces the BUN in a significant manner and importantly, the lowest amount was shown by the pre-treatment group. Further, it has been already described that lowering uric acid levels alleviates renal dysfunction[58]. In the present study, treatment with febuxostat reduced the amount of uric acid comparatively which was elevated in the case of treatment with gentamicin. The aforesaid key findings of the present study certainly suggest that febuxostat might possess a renal-protective potential against experimentally induced gentamicin-nephrotoxicity.

Subsequently, the level of electrolytes was also determined as the amount of electrolyte might provide valuable information about the renal function and should be a part of the required assessment of ARF[59]. As part of this, sodium, potassium and calcium levels were determined in this experimental study. The amount is elevated in renal failure, thereby gentamicin increases the concentration of the electrolyte. Although the amount was followed a downward trend in the case of febuxostat, pre-treatment of febuxostat reduced the amount significantly as the level was less than that of post-treatment with febuxostat.

Furthermore, the histopathological study of the kidney delineated that gentamicin significantly damaged the tubular cell of the rat kidney that ultimately leading to tubular necrosis and corroborated the nephrotoxic effect induced by gentamicin. However, treatment with febuxostat showed lesser damage as compared to the treatment with gentamicin. Hence, the result of the present study further proposes that febuxostat might possess a potential renal protective activity.

The study shows that early assessment of Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) plays a key role in drug discovery. Presently, *in silico* methods have been commonly used to estimate the ADMET properties because *in vivo* and *in vitro* testing is expensive and time-consuming[41,60]. According to Lipinski's rule, orally administered drugs must have a molecular weight of 500 (<500 g mol^{-1}), ten hydrogen bond acceptor sites (<10), five hydrogen bond donor sites (<5) and a Lipophilicity value of LogP of five ($\leq$5). If any of these laws are broken, the drug or compound would not be deemed to have good oral bioavailability[61]. This study demonstrated that febuxostat followed these rules, which indicate good oral bioavailability. The use of admetSAR in *in silico* toxicology analysis of febuxostat showed that it is non-Ames, non-carcinogenic and has low rat toxicity properties.

CONCLUSION

Based on the discussion, it can be concluded that febuxostat might have the potential to prevent gentamicin-induced experimental nephrotoxicity. Based on the biochemical and histopathological outcomes as noted in the present study, administration of febuxostat can rescue the cell from the gentamicin-nephrotoxic effect. The present study, therefore, proposes that febuxostat might be a potential pharmacologic intervention against gentamicin-induced nephrotoxicity.

SIGNIFICANCE STATEMENT

Drug-induced nephrotoxicity is a common cause of acute kidney injury. There is still a lack of extensive experimental studies investigating the renal protective potential of febuxostat in animal models. In this study, treatment with febuxostat in experimental rats showed protection against gentamicin-induced nephropathy. This is probably due to the relief of inflammation and oxidative stress associated with nephrotoxicity. The use of selective xanthine oxidase inhibitor, febuxostat offers new scope for potential nephroprotective treatment against drug-induced acute kidney injury.

ACKNOWLEDGMENTS

The authors are grateful to the Deanship of Scientific Research at King Khalid University for funding this study through the Large Research Group Project, under grant number RGP2/186/42.

REFERENCES

1. Rosenberg, C.R., X. Fang and K.R. Allison, 2020. Potentiating aminoglycoside antibiotics to reduce their toxic side effects. PLoS ONE, Vol. 15. 10.1371/journal.pone.0237948.
2. Udupa, V. and V. Prakash, 2019. Gentamicin induced acute renal damage and its evaluation using urinary biomarkers in rats. Toxicol. Rep., 6: 91-99.
3. Medić, B., M. Stojanović, B. Rovčanin, D. Kekić and S.R. Škodrić *et al*., 2019. Pioglitazone attenuates kidney injury in an experimental model of gentamicin-induced nephrotoxicity in rats. Sci. Rep., Vol. 9. 10.1038/s41598-019-49835-1.
4. Al-Azzam, S.I., K.K. Abdul-Razzak and M.W. Jaradat, 2010. The nephroprotective effects of pioglitazone and glibenclamide against gentamicin-induced nephrotoxicity in rats: A comparative study. J. Chemother., 22: 88-91.
5. Sun, B., X. Zhou, Z. Qu, L. Sun and G. Cheng *et al*., 2019. Urinary biomarker evaluation for early detection of gentamycin-induced acute kidney injury. Toxicol. Lett., 300: 73-80.
6. Balakumar, P., A. Rohilla and A. Thangathirupathi, 2010. Gentamicin-induced nephrotoxicity: Do we have a promising therapeutic approach to blunt it? Pharmacol. Res., 62: 179-186.
7. El-Moduem, M., G. Laurent, M.P. Mingeot-Leclercq, H.S. Taper, J. Cumps and P.M. Tulkens, 2000. Apoptosis in renal proximal tubules of rats treated with low doses of aminoglycosides. Antimicrob. Agents Chemother., 44: 665-675.
8. Pakfetrat, Z., S. Janfeshan, F. Masjedi, M. Rafiei and Z. Karimi, 2021. Involvement of oxidative stress and toll-like receptor-4 signaling pathways in gentamicin-induced nephrotoxicity in male Sprague Dawley rats. Drug Chem. Toxicol., 19: 1-8.
9. Fan, X., W. Gu, Y. Gao, N. Ma, C. Fan and X. Ci, 2021. Daphnetin ameliorated GM-induced renal injury through the suppression of oxidative stress and apoptosis in mice. Int. Immunopharmacol., Vol. 96. 10.1016/j.intimp.2021.107601.
10. Tomşa, A., A. Răchişan, S. Pandrea, A. Benea, A. Uifălean, A. Parvu and L. Junie, 2021. Accelerated lipid peroxidation in a rat model of gentamicin nephrotoxicity. Exp. Ther. Med., Vol. 22. 10.3892/etm.2021.10652.
11. Abdel-Fattah, M.M., A.N.A.M. Elgendy and W.R. Mohamed, 2021. Xanthenone, ACE2 activator, counteracted gentamicin-induced nephrotoxicity in rats: Impact on oxidative stress and ACE2/Ang-(1–7) signaling. Life Sci., Vol. 275. 10.1016/j.lfs.2021.119387.
12. Babaeenezhad, E., N. Nouryazdan, M. Nasri, H. Ahmadvand and M.M. Sarabi, 2021. Cinnamic acid ameliorate gentamicin-induced liver dysfunctions and nephrotoxicity in rats through induction of antioxidant activities. Heliyon, Vol. 7. 10.1016/j.heliyon.2021.e07465.
13. Manikandan, R., M. Beulaja, R. Thiagarajan, A. Priyadarsini, R. Saravanan and M. Arumugam, 2011. Ameliorative effects of curcumin against renal injuries mediated by inducible nitric oxide synthase and nuclear factor kappa B during gentamicin-induced toxicity in Wistar rats. Eur. J. Pharmacol., 670: 578-585.
14. Randjelovic, P., S. Veljkovic, N. Stojiljkovic, L. Jankovic-Velickovic, D. Sokolovic, M. Stoiljkovic and I. Ilic, 2012. Salicylic acid attenuates gentamicin-induced nephrotoxicity in rats. Sci. World J., Vol. 2012. 10.1100/2012/390613.
15. El-Kashef, D.H., A.E. El-Kenawi, M.A. Rahim, G.M. Suddek and H.A. Salem, 2016. Agmatine improves renal function in gentamicin-induced nephrotoxicity in rats. Can. J. Physiol. Pharmacol., 94: 278-286.
16. Okamoto, K., B.T. Eger, T. Nishino, S. Kondo, E.F. Pai and T. Nishino, 2003. An extremely potent inhibitor of xanthine oxidoreductase. J. Biol. Chem., 278: 1848-1855.
17. Faruque, L.I., A. Ehteshami-Afshar, N. Wiebe, L. Tjosvold, J. Homik and M. Tonelli, 2013. A systematic review and meta-analysis on the safety and efficacy of febuxostat versus allopurinol in chronic gout. Semin. Arthritis Rheumatism, 43: 367-375.
18. Liu, X., K. Liu, Q. Sun, Y. Wang, J. Meng, Z. Xu and Z. Shi, 2018. Efficacy and safety of febuxostat for treating hyperuricemia in patients with chronic kidney disease and in renal transplant recipients: A systematic review and meta-analysis. Exp. Ther. Med., 16: 1859-1865.

19. Fahmi, A.N.A., G.S.G. Shehatou, A.M. Shebl and H.A. Salem, 2016. Febuxostat protects rats against lipopolysaccharide-induced lung inflammation in a dose-dependent manner. Naunyn-Schmiedeberg's Arch. Pharmacol., 389: 269-278.
20. Maresh, M.M., R.R. Abdelaziz and T.M. Ibrahim, 2020. Febuxostat mitigates concanavalin A-induced acute liver injury *via* modulation of MCP-1, IL-1β, TNF-α, neutrophil infiltration, and apoptosis in mice. Life Sci., Vol. 260. 10.1016/j.lfs.2020.118307.
21. Kimura, K., T. Hosoya, S. Uchida, M. Inaba and H. Makino *et al*., 2018. Febuxostat therapy for patients with stage 3 CKD and asymptomatic hyperuricemia: A randomized trial. Am. J. Kidney Dis., 72: 798-810.
22. Bardin, T. and P. Richette, 2019. The role of febuxostat in gout. Curr. Opin. Rheumatol., 31: 152-158.
23. Lee, J.W. and K.H. Lee, 2019. Comparison of renoprotective effects of febuxostat and allopurinol in hyperuricemic patients with chronic kidney disease. Int. Urol. Nephrol., 51: 467-473.
24. Kim, S., H.J. Kim, H.S. Ahn, S.W. Oh and K.H. Han *et al*., 2017. Renoprotective effects of febuxostat compared with allopurinol in patients with hyperuricemia: A systematic review and meta-analysis. Kidney Res. Clin. Pract., 36: 274-281.
25. Kwak, C.H., M. Sohn, N. Han, Y.S. Cho, Y.S. Kim and J.M. Oh, 2018. Effectiveness of febuxostat in patients with allopurinol-refractory hyperuricemic chronic kidney disease. Int. J. Clin. Pharmacol. Ther., 56: 321-327.
26. Chewcharat, A., Y. Chen, C. Thongprayoon, A.M. Harrison, M.A. Mao and W. Cheungpasitporn, 2021. Febuxostat as a renoprotective agent for treatment of hyperuricaemia: A meta analysis of randomised controlled trials. Int. Med. J., 51: 752-762.
27. Fahmi, A.N.A., G.S.G. Shehatou, A.M. Shebl and H.A. Salem, 2016. Febuxostat exerts dose-dependent renoprotection in rats with cisplatin-induced acute renal injury. Naunyn-Schmiedeberg's Arch. Pharmacol., 389: 819-830.
28. Komers, R., B. Xu, J. Schneider and T.T. Oyama, 2016. Effects of xanthine oxidase inhibition with febuxostat on the development of nephropathy in experimental type 2 diabetes. Br. J. Pharmacol., 173: 2573-2588.
29. Khames, A., M.M. khalaf, A.M. Gad and O.M.A. El-Raouf, 2017. Ameliorative effects of sildenafil and/or febuxostat on doxorubicin-induced nephrotoxicity in rats. Eur. J. Pharmacol., 805: 118-124.
30. Lee, H.J., K.H. Jeong, Y.G. Kim, J.Y. Moon and S.H. Lee *et al*., 2014. Febuxostat ameliorates diabetic renal injury in a streptozotocin-induced diabetic rat model. Am. J. Nephrol., 40: 56-63.
31. Fukui, T., M. Maruyama, K. Yamauchi, S. Yoshitaka, T. Yasuda and Y. Abe, 2015. Effects of febuxostat on oxidative stress. Clin. Ther., 37: 1396-1401.
32. Steubl, D. and M.C. Michel, 2016. Experimental and clinical nephroprotection by the xanthine oxidase inhibitor febuxostat. Naunyn-Schmiedeberg's Arch. Pharmacol., 389: 815-817.
33. Alshahawey, M., S.M. Shaheen, T. Elsaid and N.A. Sabri, 2019. Effect of febuxostat on oxidative stress in hemodialysis patients with endothelial dysfunction: A randomized, placebo-controlled, double-blinded study. Int. Urol. Nephrol., 51: 1649-1657.
34. Sircar, D., S. Chatterjee, R. Waikhom, V. Golay, A. Raychaudhury, S. Chatterjee and R. Pandey, 2015. Efficacy of febuxostat for slowing the GFR decline in patients with CKD and asymptomatic hyperuricemia: A 6-month, double-blind, randomized, placebo-controlled trial. Am. J. Kidney Dis., 66: 945-950.
35. Tanaka, K., M. Nakayama, M. Kanno, H. Kimura and K. Watanabe *et al*., 2015. Renoprotective effects of febuxostat in hyperuricemic patients with chronic kidney disease: A parallel-group, randomized, controlled trial. Clin. Exp. Nephrol., 19: 1044-1053.
36. Khaksari, M., S. Esmaili, R. Abedloo and H. Khastar, 2021. Palmatine ameliorates nephrotoxicity and hepatotoxicity induced by gentamicin in rats. Arch. Physiol. Biochem., 127: 273-278.
37. Omori, H., N. Kawada, K. Inoue, Y. Ueda and R. Yamamoto *et al*., 2012. Use of xanthine oxidase inhibitor febuxostat inhibits renal interstitial inflammation and fibrosis in unilateral ureteral obstructive nephropathy. Clin. Exp. Nephrol., 16: 549-556.
38. Elsisi, A.E.E., S.S. Sokar, M.F. Shalaby and S.E.S. Abu-Risha, 2021. Nephroprotective effects of febuxostat and/or mirtazapine against gentamicin-induced nephrotoxicity through modulation of ERK 1/2, NF-κB and MCP1. Exp. Rev. Clin. Pharmacol., 14: 1039-1050.
39. Kent, A.L., L.E. Maxwell, M.E. Koina, M.C. Falk, D. Willenborg and J.E. Dahlstrom, 2007. Renal glomeruli and tubular injury following indomethacin, ibuprofen and gentamicin exposure in a neonatal rat model. Pediatr. Res., 62: 307-312.
40. Daina, A., O. Michielin and V. Zoete, 2017. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep., Vol. 7. 10.1038/srep42717.
41. Yang, H., C. Lou, L. Sun, J. Li and Y. Cai *et al*., 2019. AdmetSAR 2.0: Web-service for prediction and optimization of chemical ADMET properties. Bioinformatics, 35: 1067-1069.
42. Lopez-Novoa, J.M., Y. Quiros, L. Vicente, A.I. Morales and F.J. Lopez-Hernandez, 2011. New insights into the mechanism of aminoglycoside nephrotoxicity: An integrative point of view. Kidney Int., 79: 33-45.
43. Bove, M., A.F.G. Cicero and C. Borghi, 2017. The effect of xanthine oxidase inhibitors on blood pressure and renal function. Curr. Hypertens. Rep., Vol. 19. 10.1007/s11906-017-0793-3.

44. Kielstein, J.T., R. Pontremoli and M. Burnier, 2020. Management of hyperuricemia in patients with chronic kidney disease: A focus on renal protection. Curr. Hypertens. Rep., Vol. 22. 10.1007/s11906-020-01116-3.
45. Frezzatti, R. and P.F. Silveira, 2011. Allopurinol reduces the lethality associated with acute renal failure induced by *Crotalus durissus terrificus* snake venom: Comparison with probenecid. PLoS Negl. Trop. Dis., Vol. 5. 10.1371/journal.pntd.0001312.
46. Biagioni, E., M. Marietta, S. Busani, L. Rinaldi and M. Girardis, 2012. Acute renal failure and liver necrosis associated to allopurinol therapy. Anaesth. Intensive Care., 40: 190-192.
47. Hu, A.M. and J.N. Brown, 2020. Comparative effect of allopurinol and febuxostat on long-term renal outcomes in patients with hyperuricemia and chronic kidney disease: A systematic review. Clin. Rheumatol., 39: 3287-3294.
48. Yang, A.Y., 2020. Comparison of long-term efficacy and renal safety of febuxostat and allopurinol in patients with chronic kidney diseases. Int. J. Clin. Pharmacol. Ther., 58: 21-28.
49. Malik, U.Z., N.J. Hundley, G. Romero, R. Radi, B.A. Freeman, M.M. Tarpey and E.E. Kelley, 2011. Febuxostat inhibition of endothelial-bound XO: Implications for targeting vascular ROS production. Free Radical Biol. Med., 51: 179-184.
50. Sezai, A., M. Soma, K.I. Nakata, S. Osaka and Y. Ishii *et al*., 2015. Comparison of febuxostat and allopurinol for hyperuricemia in cardiac surgery patients with chronic kidney disease (NU-FLASH trial for CKD). J. Cardiol., 66: 298-303.
51. Omizo, H., Y. Tamura, C. Morimoto, M. Ueno and Y. Hayama *et al*., 2020. Cardio-renal protective effect of the xanthine oxidase inhibitor febuxostat in the 5/6 nephrectomy model with hyperuricemia. Sci. Rep., Vol. 10. 10.1038/s41598-020-65706-6.
52. Lin, T.C., L.Y. Hung, Y.C. Chen, W.C. Lo, C.H. Lin, K.W. Tam and M.Y. Wu, 2019. Effects of febuxostat on renal function in patients with chronic kidney disease: A systematic review and meta-analysis. Medicine, Vol. 98. 10.1097/md.0000000000016311.
53. Tsuda, H., N. Kawada, J.Y. Kaimori, H. Kitamura and T. Moriyama *et al*., 2012. Febuxostat suppressed renal ischemia-reperfusion injury via reduced oxidative stress. Biochem. Biophys. Res. Commun., 427: 266-272.
54. Edeogu, C.O., M.E. Kalu, A.C. Famurewa, N.T. Asogwa, G.N. Onyeji and K.O. Ikpemo, 2020. Nephroprotective effect of *Moringa oleifera* seed oil on gentamicin-induced nephrotoxicity in rats: Biochemical evaluation of antioxidant, anti-inflammatory, and antiapoptotic pathways. J. Am. Coll. Nutr. 39: 307-315.
55. Sharma, V.C., A. Kaushik, Y.N. Dey, B. Srivastava, M. Wanjari, S. Pawar and S. Chougule, 2021. Nephroprotective potential of *Anogeissus latifolia* Roxb. (Dhava) against gentamicin-induced nephrotoxicity in rats. J. Ethnopharmacol., Vol. 273. 10.1016/j.jep.2021.114001.
56. Vega, M.W., S.J. Swartz, S. Devaraj and S. Poyyapakkam, 2020. Elevated serum creatinine: But is it renal failure? Pediatrics, Vol. 146. 10.1542/peds.2019-2828.
57. Gowda, S., P.B. Desai, S.S. Kulkarni, V.V. Hull, A.A.K. Math and S.N. Vernekar, 2010. Markers of renal function tests. N. Am. J. Med. Sci., 2: 170-173.
58. Giordano, C., O. Karasik, K. King-Morris and A. Asmar, 2015. Uric acid as a marker of kidney disease: Review of the current literature. Dis. Markers, Vol. 2015. 10.1155/2015/382918.
59. Wu, H. and J. Huang, 2018. Drug-induced nephrotoxicity: Pathogenic mechanisms, biomarkers and prevention strategies. Curr. Drug Metab., 19: 559-567.
60. Cheng, F., W. Li, G. Liu and Y. Tang, 2013. *In silico* ADMET prediction: Recent advances, current challenges and future trends. Curr. Top. Med. Chem., 13: 1273-1289.
61. Lipinski, C.A., F. Lombardo, B.W. Dominy and P.J. Feeney, 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Delivery Rev., 46: 3-26.