Hesperetin attenuates teicoplanin-induced acute kidney injury by mitigating oxidative stress and inflammation in Wistar rats

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Abstract

Objectives: Renal failure has been reported in patients treated with teicoplanin (TEIC), a widely used antibiotic. Hesperetin, a flavonoid in citrus fruits, has been reported to possess nephroprotective effects. This study investigated the protective effect of hesperetin on TEIC-induced nephrotoxicity in rats.

Methods: Male Wistar rats (n = 32, 144–180 g) were grouped into four groups of eight rats each. The control, group 1 received water, group 2 received 50 mg/kg of hesperetin orally, group 3 received 10 mg/kg of TEIC intraperitoneally and group 4 received 50 mg/kg of hesperetin and 10 mg/kg of TEIC. Administration was done for 21 days. Rats were sacrificed 24 h after the last administration, and the kidney was excised and used for biochemical assays.

Results: Administration of TEIC resulted in kidney injury that was characterized by significant increase in plasma urea and creatinine level relative to control group (P < 0.05). Additionally, a significant increase in the biomarkers of inflammation (NO, myeloperoxidase, TNF-α) and lipid peroxidation (malondialdehyde) of TEIC-treated rats was observed when compared to the control group. Furthermore, altered antioxidant status was observed following TEIC treatment. Activities of enzymic antioxidants (SOD, CAT, GPx, GST) and concentration of non-enzymic antioxidants (GSH and ascorbic acid) were downregulated. In comparison to the TEIC-treated group, the administration of hesperetin with TEIC significantly reduced all changes in the markers of kidney injury, inflammation and oxidative stress. Histopathological examination revealed that rats given TEIC showed increased glomerular size and considerable hydropic changes in the proximal convoluted tubules, whereas rats given HESP and TEIC showed only mildly enhanced glomerular size and hydropic modifications.

Conclusion: Hesperetin preserved the histo-architecture of the kidney. From this study, hesperetin offered a protective effect against TEIC-induced nephrotoxicity in rats.

Significance statement

One of the known hazards of TEIC, a frequently used antibiotic, has been renal failure, among others. This study sheds fresh light on the efficacy of hesperetin, a flavonoid found in many citrus fruits, for preventing TEIC-induced kidney damage by reducing oxidative stress and inflammation. Also, hesperetin should be considered as an alternate supplement against TEIC-induced kidney impairment. Furthermore, the data reported in this study will provide useful information for future research into the therapeutic benefits of hesperetin as an alternative therapy for renal damage.

Introduction

The advent of antibiotics gave the healthcare community hope of victory against infectious diseases; however, global problems of infectious and deadly diseases caused by bacteria are currently major scientific and medical issues. A rapidly growing problem with potentially devastating consequences is bacterial resistance to antimicrobials generated by a wide range of antibiotics, including glycopeptides (Reygaert 2018).

Glycopeptides are referred to as a class of antibiotics of last resort for the treatment of multidrug-resistant human pathogens such as Staphylococcus aureus, Enterococcus spp., and Clostridium difficile infections. The first generation of glycopeptide antibiotics is vancomycin and teicoplanin (TEIC) (Jovetic et al. 2010, Rossolini et al. 2014). The chemical structure of teicoplanin is provided in Fig. 1. TEIC inhibits cell wall synthesis by binding to the acyl-d-alanyl-d-alanine terminus of the developing peptidoglycan on the cytoplasmatic membrane’s outer side (Jovetic et al. 2010). TEIC has been known to cause a number of toxicities such as nephrotoxicity and neurotoxicity among others and adverse effects in experimental animals and humans. Some of its reported toxicity in animal models and humans

Hesperetin (HESP), a bioactive product of degradation of hesperidin, is prevalent in Citrus aurantium fruit peel. The chemical structure of hesperetin is given in Fig. 2. It has been the subject of several in vivo and in vitro studies, all of which have yielded positive results. HESP is said to possess antioxidant, anti-inflammatory, anti-ototoxic, anticarcinogenic, antiatherogenic, and nephroprotective effects among other biological activities (Pari & Shagirtha 2012, Parhiz et al. 2015, Olayinka et al. 2022). HESP has demonstrated free radical scavenging activity, inhibited pro-inflammatory cytokines, cyclooxygenases, and anti-inflammatory efficacy (Kumar et al. 2017). It has also been demonstrated to possess neuroprotective, nephroprotective and hepatoprotective effect (Sangpheak et al. 2015, Kumar et al. 2017, Kheradmand et al. 2018, Tabeshpour et al. 2020). Given this information about HESP, this study thus aimed to investigate the protective effect of HESP on TEIC-induced nephrotoxicity in rats.

### Materials and methods

#### Chemicals and reagents

TEIC (Targocid\textsuperscript{\textregistered}) is a product of Sanofi Aventis Paris, France, and HESP was from AK Scientific, USA. Urea and creatinine kits were products from RANDOX Laboratories, United Kingdom. ELISA kits were obtained from Biolegend USA. Ellman’s reagent (5’-5’-dithiobis-(2-dinitrobenzoic acid), sulfosalicyclic acid, di-potassium orthophosphate, potassium di-hydrogen orthophosphate, BSA, thiobarbituric acid (TBA), glutathione and 1-chloro-2,4-dinitrobenzene and all additional reagents were purchased from Sigma–Aldrich and were of analytical grade.

#### Experimental animals and treatment

For this investigation, 32 healthy male Wistar rats weighing between 144 and 190 g were employed. The animals were purchased from the Central Animal House at the University of Ibadan College of Medicine. The rats were confined in cages made of plastic at the animal house, Ajayi Crowther University, Oyo, for the period of acclimatization and treatment. The rats were acclimatized for a period of 1 week and were allowed free access to food and water. Handling of the experimental animals was consistent with international principles on the care and use of experimental animals (NRC 2011). The Ethical Review Committee of Ajayi Crowther University’s Faculty of Natural Sciences provided approval and permission to use the animals with ethical number FNS/ERC/2021/130H. The rats were divided into four groups, each of eight rats: group 1 (control) received water, group 2 (HESP ONLY) received HESP (50 mg/kg body weight) orally, group 3 received TEIC (10 mg/kg body weight) intraperitoneally, while group 4 (TEIC+HESP) received HESP orally (50 mg/kg body weight) and TEIC (10 mg/kg body weight)
intraperitoneally immediately after HESP administration. All treatments lasted for a period of 21 days. Normal saline was used as vehicle for HESP and TEIC.

**Collection of blood and preparation of plasma**

Whole blood was collected from the retro-orbital venous plexus of the animals into vials containing heparin and plasma was obtained by centrifuging the blood at 1792 g for 10 min.

**Sacrification of experimental animals**

The experimental animals were left for 24 h after the last administration and sacrificed by cervical dislocation afterward. Animals were rapidly dissected to excise the kidney, rinsed in ice-cold 1.15% KCL, blotted and weighed. Kidney collected was homogenized in five volumes/weight of ice cold 0.1 M phosphate buffer of pH 7.4. The homogenate was centrifuged for 15 min at 10,000 g at 4°C, and the supernatant collected was stored at −4°C and used for subsequent biochemical assays. The kidney was fixed in 4% phosphate-buffered formalin and further processed for histology.

**Assay of biomarkers of kidney function**

The concentrations of creatinine and urea in plasma were measured using a creatinine and urea kit respectively according to the manufacturer's instructions.

**Assay of biomarkers of oxidative imbalance of redox state**

Activity of superoxide dismutase (SOD) in tissue homogenate was measured as described by Sun and Zigman (1978). At an alkaline medium pH 10.2, the inhibition of epinephrine autooxidation was observed. The amount of SOD required to generate a 50% inhibition of the oxidation of adrenaline to adrenochrome during a period of 1 minute is considered one unit of SOD activity.

Catalase (CAT) activity in tissue homogenate was assayed according to the method described by Claiborne (1985). In brief, the assay combination contained 1 mL of 30 mM H$_2$O$_2$, 1.9 mL of 50 mM phosphate buffer, and 0.1 mL of supernatant tissue homogenate. Spectrophotometric measurements at 240 nm were used to constantly track the oxidation of H$_2$O$_2$ for 60 s. The CAT activity was measured by the variation in absorbance, which was given as unit/mg protein.

The level of reduced glutathione (GSH) in the tissue homogenate was determined by the method described by Jollow et al. (1974). Ellman's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid) and reduced glutathione combine to produce a chromophoric compound with a molar absorbance at 412 nm.

Glutathione-S-transferase (GST) activity was determined using method described by Habig et al. (1974). Briefly, the assay combination contained 3.0 mL of renal post mitochondrial fraction (PMF), 2.79 mL of phosphate buffer (0.1 M, pH 6.5), 150 μL of 2,4-dinitrochlorobenzene (CDNB) (3.37 mg/mL) and 30 μL of reduced GSH (0.1 M). The reaction was allowed to stabilize for 60 s before the absorbance was measured at 340 nm in comparison to the blank.

The concentration of ascorbic acid (AA) in tissue homogenates was determined using the method described by Jagota and Dani (1982). This approach produces a blue color with a maximum absorbance at 760 nm when AA in biological samples interacts with Folin-Ciocalteu reagent.

Glutathione peroxidase (GPx) activity was determined according to the procedure explained by Rotruck et al. (1973). Phosphate buffer (0.2 mL) (0.4 M), 0.1 mL of sodium azide (10 mM), 0.2 mL of tissue homogenate and 0.2 mL of GSH were all included in the reaction media. H$_2$O$_2$ (0.2 mM) was added to the mixture to start the reaction. With the aid of Ellman's reagent, the GSH content was measured. The activity was expressed as a unit per milligram of protein, where a unit is equal to the amount of GSH that is consumed every minute.

Varshney and Kale's method was used to assess the amount of thiobarbituric acid reactive species in tissue homogenates (1990). Malondialdehyde (MDA) and thiobarbituric acid were used in the procedure to create a persistent pink chromophore with a maximum absorbance of 532 nm. Lipid peroxidation, in nmol/mg protein, was computed as:

$$\text{MDA (units/mg protein)} = (\text{absorbance} \times \text{volume of mixture})/ (\text{ES32} \times \text{volume of sample} \times \text{mg protein})$$

where ES32 is the molar extinction coefficient for MDA = 1.56 × 10$^3$ M$^{-1}$ cm$^{-1}$.

**Assay of inflammation biomarkers**

Nitric oxide concentration was determined by estimating nitrite level following the method of Green et al. (1982). Nitrite measurement is used to quantify nitric oxide (NO), one of its stable oxidation products. A pink azo color is produced when nitrite interacts with sulfanilamide and
N-(1-naphthyl) ethylenediamine dihydrochloride (NED). Utilizing its absorbance at 550 nM, the azo dye generated in this test is quantified spectrophotometrically.

Myeloperoxidase activity was measured spectrophotometrically by the method described by Kim (2012). A plate was incubated at 37°C for 5 min with 10 μL of sample, 80 μL of 0.75 mM H₂O₂ and 110 μL of TMB solution (2.9 mM TMB in 14.5% DMSO and 150 mM sodium phosphate buffer at pH 5.4). After adding 50 μL of 2M H₂SO₄, the reaction was stopped, and the myeloperoxidase (MPO) activity was calculated by measuring the absorbance at 450 nm.

Level of TNF-α was determined in the tissue homogenate using ELISA kit (Biolegend) following the manufacturer’s procedure.

**Histopathological analysis**

The fixed kidney was dehydrated with ethanol and xylene prior to getting embedded in paraffin and stained with hematoxylin and eosin before being scanned.

**Statistical analysis**

Results were expressed as mean ± s.d. Data were analyzed by subjecting data obtained by ANOVA using Graphpad Prism (V 8.01) and Tukey post hoc test. P values less than or equal to 0.05 were considered to be statistically significant.

**Results**

**Hesperetin alleviates TEIC-induced alteration in kidney function indices**

The protective effect of HESP on TEIC-induced alterations in plasma creatinine and urea in rats was shown in Fig 3. Administration of TEIC caused a significant increase in plasma levels of creatinine and urea when compared to control (P < 0.05). However, coadministration of HESP and TEIC significantly ameliorated the alterations relative to TEIC-treated group.

**Hesperetin ameliorates TEIC-induced alterations in antioxidant status**

The protective effect of HESP against TEIC-induced changes in enzymic (GPx and GST) and non-enzymic (GSH and AA) antioxidants was shown in Fig. 4. Activities of glutathione peroxidase (GPx) and glutathione-S-Transferase (GST) in the kidney of the TEIC-treated group were significantly downregulated when compared to control. Coadministration of HESP with TEIC significantly attenuated the alterations relative to TEIC group. Furthermore, administration of TEIC caused a significant decrease in the levels of GSH and AA when compared to the control (P < 0.05); however, coadministration of HESP with TEIC significantly protected against the alteration relative to TEIC group.
Hesperetin ameliorates TEIC-induced alterations in lipid peroxidation

Figure 5 shows TEIC-induced changes in MDA levels in the kidney of rats. There was significant increase in the level of MDA in the kidney of TEIC-treated animals when compared to control. However, coadministration of HESP and TEIC significantly attenuated the alteration relative to TEIC-treated group.

Hesperetin ameliorates TEIC-induced inflammation

Figure 6 presents the protective effect of HESP on TEIC-induced alterations in the levels of NO and TNF-α and activities of MPO in the kidney of rats. Administration of TEIC caused a significant increase (P<0.05) in the levels of NO and TNF-α and activities of MPO when compared to control. However, coadministration of HESP with TEIC significantly attenuated the increase in NO, TNF-α and MPO relative to TEIC-treated group.

Hesperetin ameliorates TEIC-induced histopathological alterations in the kidney of rats

Histopathological examination of the kidney of rats showed inflammation in the kidney of TEIC-treated rats relative to control. HESP preserved the histo-architecture of the kidney (Fig. 7).

Discussion

Through the production of reactive oxygen species (ROS), which can alter proteins, lipids, and DNA, redox disturbances are known to have a deleterious impact on bodily systems. Larger perfusion and higher concentrations of excreted compounds, which occur in renal tubular cells, make the kidney more susceptible to injury (Halliwell & Gutteridge 1990). TEIC, a widely used antibiotic, on the other hand, has been demonstrated to induce nephrotoxicity in experimental animals and humans (Patel et al. 2012, Sazanami et al. 2021). However, HESP is said to possess antioxidant, anti-inflammatory, and nephroprotective effects among other biological activities (Parhiz et al. 2015, Olayinka et al. 2022). The current research looked at how HESP protects against TEIC-induced nephrotoxicity.

Our data demonstrated that TEIC administration resulted in significant elevation of plasma levels of urea and creatinine. Increased urea and creatinine level in the blood has been linked to renal damage (Sazanami et al. 2021). The pattern of elevation of these markers has been shown to be vital to the diagnosis of kidney injury. The alterations in plasma level of these kidney function biomarkers induced by TEIC were ameliorated with coadministration of HESP to TEIC-intoxicated rats. Therefore, this suggests that HESP may function by reducing elevated levels of renal kidney damage indicators. The protective effect of HESP on TEIC-induced alterations in SOD and CAT activities in the kidney of rats administered with HESP and TEIC was presented in Table 1. Administration of TEIC caused a significant
(P < 0.05) decrease in SOD and CAT activities in the kidney by 68 and 50% respectively when compared to the control group. Co-administration of HESP with TEIC significantly protected against the decrease in SOD and CAT activities relative to TEIC-treated group.

Also, TEIC administration caused a significant depletion of the kidney antioxidant defense system in rats as evident by the declined enzymic and non-enzymic antioxidants. GSH and AA levels were significantly reduced, while activities of SOD, CAT, GST and GSH-Px were significantly downregulated in TEIC-treated rats. GSH and AA are non-enzymic antioxidants that defend against oxidants in vivo, while SOD dismutates superoxide radicals to hydrogen peroxide (H$_2$O$_2$) (Ajayi et al. 2022), a substrate for CAT and GSH-Px. GSH is required for GST activity and it participates in the detoxification of drugs and toxicants. Depletion in the activities of the aforementioned enzymatic antioxidants and levels of the non-enzymic antioxidants may lead to accumulation of ROS which causes oxidative imbalance of redox state and may be responsible for the observed renal injury in the TEIC-treated rats. Co-administration of HESP with TEIC alleviates oxidative imbalance of redox state induced by TEIC. Additionally, increase in MDA (a marker of oxidative damage to lipids) level was observed in TEIC-treated rats. Increased lipid peroxidation has been shown to be associated with overproduction of ROS (Olayinka et al. 2022). Co-administration of HESP alongside TEIC significantly attenuates TEIC-induced oxidative imbalance of redox state as indicated by decrease in renal MDA level.

Furthermore, biomarkers of inflammation (NO, TNF-α, and MPO) were significantly increased in the TEIC-treated rats. MPO utilizes hydrogen peroxide produced by the neutrophils to oxidize a variety of aromatic compounds to give substrate radicals for bacterial activity. Also, MPO oxidizes chloride ions to produce a strong non-radical oxidant hypochlorous acid. Excessive production of hypochlorous acid causes oxidative stress leading to tissue injury (Ndrepepa 2019). TNF-α a proinflammatory cytokine is produced by macrophages during acute inflammation. NO has been demonstrated to be a proinflammatory substance for TNF-α (Simeone et al. 2002). Increased levels of NO and TNF-α and activity of MPO in TEIC-treated rats showed that TEIC induces inflammation in the kidney of the rats which was ameliorated by coadministration of HESP. From the foregoing, it can be concluded that HESP likely mode of action against TEIC-induced kidney injury involves oxidative balance of the redox state and an anti-inflammatory effect.

Our data also demonstrated that TEIC induced histopathological changes in the kidney tissue. TEIC treatment caused increased glomerular size and hydrophobic changes in the proximal convoluted tubules in the H&E-stained sections. However, HESP administration alongside TEIC significantly improved the histological changes caused by TEIC.

### Table 1

Protective effect of HESP on TEIC-induced alterations in activities of superoxide dismutase (SOD) and catalase (CAT) in the kidney of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (unit SOD)</th>
<th>CAT (μmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.00 ± 1.83</td>
<td>11.51 ± 0.29</td>
</tr>
<tr>
<td>HESP</td>
<td>72.30 ± 2.63</td>
<td>10.45 ± 0.12</td>
</tr>
<tr>
<td>TEIC</td>
<td>24.34 ± 1.52(68%)$^a$</td>
<td>5.65 ± 0.41(50%)$^a$</td>
</tr>
<tr>
<td>HESP+TEIC</td>
<td>56.70 ± 2.06$^{a,b}$</td>
<td>9.01 ± 0.40$^{a,b}$</td>
</tr>
</tbody>
</table>

Each bar represents the mean ± s.o. (n = 8). $^a$Significantly different when compared to control (P < 0.05); $^b$Significantly different when compared to TEIC (P < 0.05). Values in parenthesis represents percentage (%) decrease. HESP, hesperetin (50 mg/kg body weight); TEIC, teicoplanin (10 mg/kg body weight).

### Conclusion

From this study, HESP offered protective effect against TEIC-induced nephrotoxicity via antioxidant and anti-inflammatory actions. HESP may be of therapeutic potential in alleviating the nephrotoxic effects of TEIC.
Declaration of interest
There are no conflicts of interest declared by the authors.

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Author contribution statement
ETO originated the work and designed the experiment. TOO carried out the assays and assisted in the analysis of the experiment. ATO and SAK assisted with the analysis and developed the manuscript. BOA assisted with the experimental design.

References

Ajayi BO, Olajide TA & Olayinka ET 2022 6-gingerol attenuates pulmonary inflammation and oxidative stress in mice model of house dust mite-induced asthma. Advances in Redox Research 5 100036. (https://doi.org/10.1016/j.ars.2022.100036)


Jollow DJ, Mitchell JR, Zampaglione N & Gillette JR 1974 Bromobenzene induced liver necrosis, Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the toxic metabolite. Pharmacology 11 151–169. (https://doi.org/10.1007/BF01136485)


Patel P, Sandoe J & Baig W 2012 Teicoplanin-induced leucopenia with immediate resolution after administration of G-CSF. BMJ Case Reports 13 bcr0120125668. (https://doi.org/10.1136/bcr.01.2012.5668)


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