

PHARMACY

Nephroprotective Effect of Luteolin Isolated from the Leaves of *Saccharum officinarum* L. against Gentamicin-Induced Nephrotoxicity in Wistar Rats: Biochemical, Gravimetric and Histopathological Evidence

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Published October 2026

Abstract

Background: Gentamicin remains an important and inexpensive aminoglycoside antibiotic, but its clinical value is limited by dose-dependent injury to the proximal tubular epithelium, a process driven largely by oxidative stress. Sugarcane (*Saccharum officinarum* L.) leaves are rich in flavonoids, including luteolin, yet the contribution of luteolin to the reported renal benefits of sugarcane has not been tested directly.

Objective: To isolate luteolin from sugarcane leaves and evaluate its nephroprotective activity against gentamicin-induced nephrotoxicity in rats.

Methods: Shade-dried leaves were extracted with ethanol in a Soxhlet apparatus, and luteolin was isolated by silica-gel column chromatography with gradient elution. Identity was supported by thin-layer chromatography against authentic luteolin, melting point, and ultraviolet and infrared spectroscopy. After an acute oral toxicity study, thirty Wistar rats were divided into five groups of six: normal control, gentamicin control (80 mg/kg, i.p., 8 days), standard antioxidant, and low-dose and high-dose luteolin-rich fraction given orally with gentamicin. Serum creatinine, blood urea nitrogen (BUN), uric acid, kidney malondialdehyde (MDA), relative kidney weight, body weight and renal histology were assessed.

Results: The ethanolic extract yield was about 9–10% w/w, and the isolate showed an R_f of 0.62 against 0.61 for standard luteolin and UV maxima near 253 and 350 nm. No mortality or toxic signs occurred in the acute study. Gentamicin raised creatinine (0.72 to 3.18 mg/dL), BUN (18.4 to 62.5 mg/dL), uric acid (1.9 to 5.8 mg/dL), kidney MDA (2.1 to 7.9 nmol/mg) and relative kidney weight (0.62 to 1.05%), and caused tubular necrosis, casts and inflammation. The high dose of luteolin-rich fraction restored these values towards normal (creatinine 1.24 mg/dL, BUN 26.8 mg/dL, MDA 3.1 nmol/mg), gave about 80% protection, and preserved tubular architecture, comparable to the standard. The low dose gave intermediate protection.

Conclusions: Luteolin from sugarcane leaves protects against gentamicin nephrotoxicity in a dose-dependent manner, most probably by suppressing lipid peroxidation. Confirmation of purity by spectroscopic methods and mechanistic studies are warranted.

Keywords: luteolin; *Saccharum officinarum*; sugarcane leaves; gentamicin; nephrotoxicity; oxidative stress; malondialdehyde; nephroprotection

1 Introduction

The kidney receives roughly a quarter of the resting cardiac output and concentrates many of the substances that pass through it, so it is exposed to high local levels of drugs and their metabolites. The proximal tubular cells, which are rich in mitochondria and transport proteins and actively take up filtered solutes, are especially vulnerable. For these reasons drug-induced nephrotoxicity is one of the commonest forms of acute kidney injury, ranging from mild, reversible falls in filtration to severe structural damage [1,2]. Established biomarkers of this injury include serum creatinine, blood urea nitrogen (BUN) and uric acid, which rise as filtration and excretion fail [2].

Gentamicin is a bactericidal aminoglycoside used widely against serious Gram-negative infections, particularly in low- and middle-income settings where it is cheap and effective. Its use is constrained by nephrotoxicity, which is a recognised clinical problem and a standard experimental model of acute kidney injury [3,4]. After glomerular filtration, gentamicin is reabsorbed by proximal tubular cells and accumulates in lysosomes and other organelles. There it disturbs mitochondrial function, promotes the generation of reactive oxygen species (ROS), triggers inflammation and activates apoptotic and necrotic pathways [3,5]. Gentamicin also reduces glomerular filtration through mesangial contraction and vasoconstriction [6]. Oxidative injury of membrane lipids, measured as malondialdehyde (MDA), is a central and consistent feature of the model [3,4]. No entirely satisfactory strategy exists to prevent this injury without withdrawing or limiting the antibiotic, which has led many investigators to test antioxidants of plant origin as adjuncts [7].

Free radicals such as the superoxide anion, hydrogen peroxide and the hydroxyl radical are produced continuously during normal metabolism and are held in check by enzymatic and non-enzymatic antioxidant defences. When production of ROS overwhelms these defences, lipids, proteins and nucleic acids are damaged, and polyunsaturated membrane lipids are particularly susceptible to a self-propagating chain of peroxidation [8]. The kidney is rich in oxidisable substrates and in mitochondria, and tubular cells exposed to a toxin such as gentamicin therefore experience oxidative stress early in the course of injury. This makes exogenous antioxidants a logical approach to protection. Plant polyphenols and flavonoids are of particular interest because they are abundant in the diet, are generally well tolerated and combine radical scavenging with effects on inflammatory and cell-survival signalling [9,10].

Sugarcane (*Saccharum officinarum* L., family Poaceae) is grown across the tropics and is known mainly for its sucrose-rich stalk. It also has a long place in traditional medicine, including use as a diuretic and in urinary disorders. Phytochemical surveys of the plant describe phenolic acids, flavonoids, sterols, saponins, tannins and other constituents [11–15]. The leaves, which are agricultural residue in most sugar-producing regions, have received comparatively little attention, although leaf extracts show antioxidant and anti-inflammatory activity [16] and a metabolomics study linked components of a leaf extract to diuretic action [17]. Other parts of the plant have been reported to be hepatoprotective [18], to affect central nervous system function [19], and to yield antioxidant anthocyanins [20]. Toxicological reports also exist, for example on the juice and reproductive function in female rats [21], which underlines the need for safety data on any preparation intended for therapeutic exploration.

Most relevant to the present work, an ethanolic extract of sugarcane leaves was reported to protect rats from gentamicin-induced nephrotoxicity [22]. A crude extract is a complex mixture, however, and the study did not identify which constituent is responsible. Luteolin (3',4',5,7-tetrahydroxyflavone) is a flavone that has been described in sugarcane leaves and is among the more potent naturally

occurring flavonoid antioxidants. Its catechol B-ring and conjugated structure allow it to donate hydrogen atoms and to scavenge radicals [9], and it also has anti-inflammatory, anti-apoptotic and cytoprotective effects [10,23,24]. In the kidney, luteolin has been shown to attenuate injury caused by cisplatin [25,26], colistin [27], mercuric chloride [28], ischaemia-reperfusion [29], lipopolysaccharide [30] and diabetes [31]. To our knowledge, however, luteolin isolated from sugarcane leaves has not been evaluated in the gentamicin model.

The present study was therefore designed to (i) isolate and characterise luteolin from the ethanolic extract of *S. officinarum* leaves, (ii) establish the acute oral safety of the material, and (iii) determine whether the luteolin-rich fraction protects against gentamicin-induced nephrotoxicity in rats, using serum markers of renal function, a marker of lipid peroxidation, relative kidney weight and histopathology, with comparison against a standard antioxidant and across two dose levels.

2 Materials and methods

2.1 Plant material and authentication

Mature, healthy, disease-free leaves of *S. officinarum* L. were collected from a single location in Himachal Pradesh. A voucher specimen including leaves and stalk was authenticated by a qualified taxonomist on the basis of its morphological characters, and a herbarium sheet was deposited under voucher number **A1B29**. Leaves were selected rather than stalk or juice because they are reported to be a source of flavonoids including luteolin [11,12].

2.2 Drying, extraction and yield

The leaves were washed with tap water, rinsed with distilled water and dried in the shade at room temperature to limit degradation of heat- and light-sensitive flavonoids. The dried material was ground to a coarse powder, sieved to obtain uniform particle size, and stored in airtight containers protected from light and moisture. A weighed quantity of powder (200 gm.) was extracted with 95% ethanol in a Soxhlet apparatus until the siphoning solvent became colourless. The extract was filtered and concentrated under reduced pressure in a rotary vacuum evaporator, then evaporated to a semi-solid mass and stored under refrigeration. Percentage yield was calculated as the weight of dry extract divided by the weight of powder taken, multiplied by 100.

2.3 Preliminary phytochemical screening

Standard qualitative tests were applied to the crude extract: the Shinoda (magnesium–hydrochloric acid) test for flavonoids, Mayer's and Dragendorff's reagents for alkaloids, the Keller–Killiani test for cardiac glycosides, ferric chloride for tannins, the froth test for saponins, the Salkowski test for steroids, Molisch's test for carbohydrates and the ninhydrin test for proteins and amino acids. Tests were repeated where necessary to exclude interfering substances.

2.4 Isolation and characterisation of luteolin

A portion of the crude extract was adsorbed on silica gel and loaded on a wet-packed column of silica gel (60–120 mesh). The column was eluted with solvents of increasing polarity, beginning with chloroform and proceeding through chloroform–methanol mixtures (9:1, 8:2 and 7:3) to methanol. Equal-volume fractions were collected and monitored by thin-layer chromatography (TLC) alongside authentic luteolin. Plates were developed in a closed chamber, examined under ultraviolet (UV) light

and the retardation factor (Rf) of each spot was calculated. Fractions giving a single spot with an Rf matching the standard were pooled and concentrated to give the luteolin-rich fraction (LRF), which was used for the biological studies.

Identity was supported by the melting point, the UV-visible absorption spectrum compared with that of authentic luteolin, and infrared (IR) spectroscopy to identify hydroxyl, conjugated carbonyl and aromatic functional groups. For quantitative estimation, a calibration curve of absorbance against concentration was constructed from standard luteolin solutions at the wavelength of maximum absorption, in accordance with the Beer-Lambert law, and used to determine the luteolin content of the isolate luteolin content of the LRF, 0.2% w/w.

2.5 Chemicals

Gentamicin sulphate injection, standard luteolin, thiobarbituric acid, silica gel, analytical-grade solvents, assay kits for creatinine, BUN and uric acid, and 10% formalin were obtained from suppliers. Normal saline (0.9% NaCl) was used as vehicle.

2.6 Experimental animals and ethics

Healthy adult Wistar albino rats of either sex weighing 150–200 g were obtained from an authorised animal facility. They were housed in polypropylene cages under controlled temperature and humidity with a 12-hour light-dark cycle, given a standard pellet diet and water *ad libitum*, and acclimatised for one week. The protocol was reviewed and approved by the Institutional Animal Ethics Committee (approval no. MP/25/B11) and all procedures followed national guidelines for the care and use of laboratory animals. The report follows the ARRIVE 2.0 recommendations [32], and the number of animals was kept to the minimum needed.

2.7 Acute oral toxicity

Acute oral toxicity of the test material was assessed according to the fixed-dose procedure of OECD Guideline 420 [33]. The material was given orally at progressively higher dose levels up to 200 mg/kg. Animals were observed frequently during the first hours and then daily for 14 days for changes in behaviour, locomotor activity, respiration, posture, skin and fur, food and water intake, and mortality. Body weight was recorded at the start and at intervals during observation. The therapeutic doses for the main study were chosen as fractions of the highest dose that produced no toxicity.

2.8 Experimental design and treatment

Thirty Wistar rats were randomly allocated to five groups of six (Table 1). Nephrotoxicity was induced in Groups II to V with gentamicin 80 mg/kg intraperitoneally once daily for eight consecutive days. Standard and test treatments were given orally each day concurrently with gentamicin. Group I received saline only. The standard was a reference antioxidant (vitamin E, dose), and the LRF was given at a low dose of 20 mg/kg and a high dose of 80 mg/kg.

Table 1. Grouping of animals and treatment schedule (n = 6 per group; 8 days).

Group	Designation	Treatment
I	Normal control	Normal saline, orally
II	Gentamicin (toxic) control	Gentamicin 80 mg/kg, i.p.
III	Standard	Gentamicin + standard antioxidant (vitamin E)

Group	Designation	Treatment
IV	Test, low dose	Gentamicin + luteolin-rich fraction, low dose, orally
V	Test, high dose	Gentamicin + luteolin-rich fraction, high dose, orally

The gentamicin model was chosen because it is widely validated, reproducible, produces tubular injury resembling that seen clinically, and is mediated substantially by oxidative stress, which matches the proposed antioxidant mechanism of luteolin [3,4].

Animals were allocated to groups by simple randomisation after acclimatisation, so that mean starting body weight was similar across groups. Cages were labelled by code, and the histopathological assessment was blinded, as described below. The eight-day gentamicin schedule was chosen because it reliably produces marked but not uniformly lethal tubular injury, and concurrent oral dosing of the protective agents was chosen to test prevention of injury, which is the clinically relevant scenario when an aminoglycoside course is planned. Oral administration of the luteolin-rich fraction reflects the route by which such a supplement would most probably be taken.

2.9 Sample collection

On the day after the last dose the animals were weighed, fasted and euthanised humanely under anaesthesia. Blood was collected, allowed to clot and centrifuged to obtain haemolysis-free serum, which was stored frozen until analysis. Both kidneys were removed, freed of fat and connective tissue, washed in ice-cold saline, blotted and weighed. One kidney was fixed in 10% formalin for histology and the other was homogenised in the cold for estimation of tissue MDA.

2.10 Biochemical estimations

Serum creatinine was measured by the alkaline picrate (Jaffe) reaction. BUN was estimated by a colorimetric urea method, and uric acid by the uricase-based enzymatic colorimetric method, all read spectrophotometrically against standards. Kidney MDA was estimated from the pink chromogen formed with thiobarbituric acid under acidic conditions and heat, essentially as described by Ohkawa and co-workers [34], and expressed as nmol/mg protein or tissue.

2.11 Body weight and relative kidney weight

The change in body weight from the beginning to the end of the study was recorded. Relative kidney weight was calculated as kidney weight divided by body weight $\times 100$. The kidneys were also examined macroscopically for size, colour, congestion and surface texture.

2.12 Histopathology

Formalin-fixed kidneys were dehydrated in graded alcohols, cleared, embedded in paraffin, sectioned with a microtome and stained with haematoxylin and eosin. Sections were examined by light microscopy by an observer blinded to group allocation. Tubular degeneration and necrosis, luminal debris and casts, inflammatory infiltration, vascular congestion and glomerular damage were graded on a scale from 0 (no injury) to 4 (severe injury), examining several fields per section.

2.13 Statistical analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM). Groups were compared by one-way analysis of variance followed by post-hoc test, e.g. Tukey or Dunnett, with each treated group

compared against the gentamicin control. Differences were considered significant at $p < 0.05$. Percentage protection for each parameter was calculated as $[(\text{Group II} - \text{treated}) / (\text{Group II} - \text{Group I})] \times 100$ from the group means.

3 Results

3.1 Extraction and phytochemical screening

Extraction gave a dark greenish-brown semi-solid mass with a yield of about 9–10% w/w of the dried powder, consistent with ethanolic extraction of leafy material. The colour reflects chlorophyll together with coloured phenolic and flavonoid constituents. Preliminary screening (Table 2) showed a strongly positive Shinoda test, confirming flavonoids, and positive tests for alkaloids, glycosides, tannins, saponins, steroids, carbohydrates and proteins. This profile agrees with published phytochemical descriptions of the plant [11,12,14].

Table 2. Preliminary phytochemical screening of the crude ethanolic leaf extract.

Constituent	Test	Result
Flavonoids	Shinoda test	Present (+)
Alkaloids	Mayer's / Dragendorff's	Present (+)
Glycosides	Keller–Killiani	Present (+)
Tannins	Ferric chloride	Present (+)
Saponins	Froth test	Present (+)
Steroids	Salkowski	Present (+)
Carbohydrates	Molisch's	Present (+)
Proteins	Ninhydrin	Present (+)

3.2 Isolation and characterisation of luteolin

Column chromatography resolved the extract into distinct coloured bands. Fractions eluted with the intermediate-polarity chloroform–methanol system gave a single well-defined TLC spot with an R_f of about 0.62, against about 0.61 for standard luteolin run under identical conditions. The isolate melted in the range reported for luteolin. Its UV spectrum showed two absorption maxima, near 253 nm and near 350 nm, the characteristic band pattern of the flavone nucleus, and its IR spectrum showed bands attributable to hydroxyl groups, a conjugated carbonyl and an aromatic system. The calibration curve of standard luteolin was linear over the concentrations used. Together these data support the identity of the isolate as luteolin, although the level of purity has not been established by high-resolution methods (see Limitations).

3.3 Acute oral toxicity

No mortality occurred at any dose level tested, and none of the animals showed changes in behaviour, locomotion, respiration, skin or fur during the 14-day observation. Body weight increased normally. The material was therefore judged to have a wide margin of safety, and the doses for the nephroprotection study were selected as fractions of the highest dose tested.

3.4 Body weight and relative kidney weight

Group I gained weight steadily, whereas the gentamicin control lost weight, indicating a systemic effect of renal injury and reduced food intake. Body weight was better preserved in the standard group and, to a similar degree, in the high-dose LRF group, with intermediate preservation at the low dose. Relative kidney weight rose from $0.62 \pm 0.03\%$ in normal animals to $1.05 \pm 0.06\%$ in the gentamicin control, reflecting swelling, congestion and inflammation of the injured organ. LRF reduced this in a dose-dependent manner (0.88% at the low dose and 0.71% at the high dose), the high dose approaching the standard ($0.74 \pm 0.04\%$) and the normal value (Table 3).

3.5 Serum markers of renal function

Gentamicin produced a marked deterioration of renal function (Table 3). Serum creatinine was more than four times the normal value (3.18 ± 0.21 versus 0.72 ± 0.05 mg/dL), BUN rose from 18.4 ± 1.2 to 62.5 ± 3.4 mg/dL, and uric acid from 1.9 ± 0.15 to 5.8 ± 0.34 mg/dL. Co-treatment with LRF significantly attenuated all three rises in a dose-dependent manner. At the high dose, creatinine was 1.24 mg/dL, BUN 26.8 mg/dL and uric acid 2.7 mg/dL, values similar to those of the standard group (1.32 ± 0.12 , 28.6 ± 2.1 and 2.9 ± 0.20 mg/dL respectively). The low dose gave intermediate values (1.86 , 41.2 and 3.9 mg/dL).

Table 3. Effect of luteolin-rich fraction (LRF) on biochemical and gravimetric parameters in gentamicin-treated rats.

Parameter	Group I Normal	Group II Gentamicin	Group III Standard	Groups IV / V LRF (low / high)
Serum creatinine (mg/ dL)	0.72 ± 0.05	3.18 ± 0.21	1.32 ± 0.12	$1.86 / 1.24$
BUN (mg/dL)	18.4 ± 1.2	62.5 ± 3.4	28.6 ± 2.1	$41.2 / 26.8$
Uric acid (mg/dL)	1.9 ± 0.15	5.8 ± 0.34	2.9 ± 0.20	$3.9 / 2.7$
Kidney MDA (nmol/mg)	2.1 ± 0.18	7.9 ± 0.42	3.4 ± 0.25	$4.9 / 3.1$
Relative kidney weight (%)	0.62 ± 0.03	1.05 ± 0.06	0.74 ± 0.04	$0.88 / 0.71$

Values are mean $\pm$ SEM (n = 6); for LRF, means are given for the low dose / high dose.

3.6 Lipid peroxidation

Kidney MDA was nearly four times higher in the gentamicin control than in normal animals (7.9 ± 0.42 versus 2.1 ± 0.18 nmol/mg), direct evidence of extensive membrane lipid peroxidation. LRF reduced MDA in a dose-dependent manner, to 4.9 nmol/mg at the low dose and 3.1 nmol/mg at the high dose, the latter close to the normal value and to the standard (3.4 ± 0.25 nmol/mg).

3.7 Percentage protection

Percentage protection calculated from the group means is shown in Table 4. The high dose of LRF gave protection of about 79–83% across creatinine, BUN, uric acid, MDA and relative kidney weight, marginally above the standard (about 72–78%), whereas the low dose gave about 40–54%. The similarity of protection across parameters suggests that the treatment acted on a common upstream cause of injury.

Table 4. Percentage protection against the gentamicin-induced changes, calculated from group means.

Parameter	Standard (III)	LRF low dose (IV)	LRF high dose (V)
Serum creatinine	76%	54%	79%
BUN	77%	48%	81%
Uric acid	74%	49%	79%
Kidney MDA	78%	52%	83%
Relative kidney weight	72%	40%	79%

3.8 Histopathology

Kidneys of normal animals showed intact tubules with clear lumina and regular epithelium, and healthy glomeruli. The gentamicin control showed extensive injury with degeneration and necrosis of tubular epithelial cells, tubular dilatation, cellular debris and casts in the lumina, inflammatory cell infiltration and vascular congestion. The standard and high-dose LRF groups showed largely preserved tubular architecture with minimal degeneration and little debris or inflammation, and the low-dose group showed intermediate protection. Injury scores followed the same order: highest in the gentamicin control, significantly lower in treated groups, and approaching normal at the high dose.

3.9 Summary of results

In summary, luteolin was isolated from the ethanolic extract of sugarcane leaves and was non-toxic in the acute study. Gentamicin produced the expected pattern of renal injury. The luteolin-rich fraction reduced the elevations of creatinine, BUN, uric acid and MDA, moved relative kidney weight and body weight towards normal, and preserved renal histology. Each effect was dose-dependent, and the effect of the high dose was comparable to that of the standard antioxidant. Biochemical, gravimetric and histological measurements agreed with one another.

4 Discussion

4.1 The gentamicin model and the extent of injury

The gentamicin control group reproduced the pattern of injury described in the literature: a several-fold rise in creatinine and BUN, hyperuricaemia, kidney enlargement, weight loss, raised MDA and tubular necrosis with casts and inflammation [3,4]. The model therefore behaved as intended, and changes seen in the treated groups can reasonably be read as modification of a genuine injury rather than of a weak or variable one. The relative kidney weight rose despite injury because acute damage causes oedema, vascular congestion and inflammatory infiltration, and its reversal by LRF is consistent with reduced swelling and inflammation.

4.2 Preservation of renal function

Creatinine is freely filtered and produced at a fairly constant rate, so its rise reflects a fall in glomerular filtration, while BUN reflects retention of nitrogenous waste [2]. That LRF lowered both in parallel, and that the high dose approached the standard, indicates that filtration was preserved. Gentamicin reduces filtration in part through mesangial contraction and reduced glomerular blood flow, in addition to its tubular effects [6], so protection of filtration may reflect benefit at both sites.

The fall in uric acid is informative in two ways: it reflects better excretory function, and because uric acid formation is linked to xanthine oxidase, which itself generates ROS, it is compatible with a less oxidising renal environment. Serum uric acid is affected by several non-renal factors, however, and should be interpreted alongside creatinine and BUN rather than alone.

4.3 Suppression of lipid peroxidation as the likely mechanism

The most mechanistically informative result is the marked, dose-dependent reduction in kidney MDA. MDA is a stable end product of the attack of ROS on polyunsaturated membrane lipids, and its accumulation in the gentamicin group is a chemical footprint of the oxidative assault that damages tubular cell membranes and organelles [3,8]. The high dose returned MDA close to normal and to the standard. Because MDA, creatinine, BUN and histology improved together, the data are consistent with the interpretation that luteolin protects the kidney principally by limiting oxidative damage. The similar percentage protection across parameters (Table 4) supports the idea that a single upstream process was affected, although similarity of percentages is suggestive rather than proof.

Antioxidant enzymes and glutathione were not measured here. Gentamicin is known to deplete superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione as they are consumed neutralising excess radicals [3,4]. It is plausible that luteolin acts both by scavenging radicals directly and by supporting these endogenous defences, and the work of others points in this direction. In a mercuric chloride model, dietary luteolin activated Nrf2-mediated signalling, the master regulator of the antioxidant response [28]. However, this was not tested in our animals, so the proposed mechanism remains inferred.

4.4 Structural basis of the antioxidant activity

The potency of luteolin is consistent with its structure. The antioxidant capacity of a flavonoid depends largely on the number and arrangement of its hydroxyl groups, and the ortho-dihydroxy (catechol) arrangement on the B-ring, together with the 2,3 double bond conjugated with the 4-oxo group, favours hydrogen donation and stabilisation of the resulting radical [9]. This explains why luteolin is generally considered more effective than flavonoids lacking these features and provides a chemical rationale for the strong protection seen at the higher dose.

4.5 Contribution of anti-inflammatory and anti-apoptotic actions

Oxidative stress, inflammation and cell death are interlinked in gentamicin injury [5]. Luteolin has broad anti-inflammatory activity [10], and in other kidney models it lowered inflammatory cytokines and reduced tubular apoptosis. In cisplatin nephrotoxicity it limited platinum accumulation, inflammation and apoptosis [25], and it regulated p53-dependent tubular apoptosis [26]. It was protective in lipopolysaccharide-induced renal injury [30], ischaemia-reperfusion [29], colistin nephrotoxicity [27] and streptozotocin-induced diabetic nephropathy [31]. The reduction in inflammatory infiltration and casts in our histological sections is compatible with these additional actions. It is probable that protection resulted from combined antioxidant and anti-inflammatory effects rather than from a single mechanism, but inflammatory and apoptotic markers were not measured here.

4.6 Relation to earlier work on sugarcane and other agents

Dewi and colleagues showed that an ethanolic extract of sugarcane leaves protected rats against gentamicin nephrotoxicity [22]. The present findings extend that observation by identifying luteolin, isolated from the same plant part, as an active constituent capable of reproducing a substantial part

of the effect. Moving from a crude extract to a defined compound is useful because a single compound can be standardised and quantified, whereas the composition of a crude extract varies with cultivar, season and processing [11,12]. The result is also in keeping with the antioxidant and anti-inflammatory activity of sugarcane leaf extracts [16] and with the diuretic effect linked to leaf constituents [17], which could contribute to renal outflow and clearance of toxicants, although this was not examined.

The magnitude of protection seen here is comparable to that reported for other natural antioxidants in the same model. Curcumin, for example, reduced gentamicin-induced oxidative stress, endoplasmic reticulum stress and apoptosis in rat kidney [7]. Direct comparison between studies is not possible because of differences in doses, durations and assays, but the convergence of results supports the general principle that antioxidant strategies can blunt gentamicin toxicity. Importantly, the high dose of LRF performed as well as the standard antioxidant in the present study, so luteolin compares favourably with an established reference agent.

4.7 Dose-dependence, comparison with the standard, and isolation route

A graded response to dose is one of the strongest arguments that an observed benefit is pharmacological rather than accidental. In every parameter measured, the low dose of LRF gave partial protection of roughly half the maximum change and the high dose gave close to four-fifths, with the same rank order in biochemistry, kidney weight and histology. This regularity is unlikely to arise by chance in randomly allocated groups. The high dose performed marginally better than the standard antioxidant in terms of percentage protection, but the numerical difference is small, and with six animals per group it should be interpreted as equivalence rather than superiority. Head-to-head testing with a larger sample and a wider range of doses would be needed to define the dose-response relationship and to estimate a median effective dose.

The choice of extraction and isolation route also deserves comment. Ethanol is a polar, relatively non-toxic solvent that dissolves flavonoid aglycones and glycosides efficiently, and its easy removal under reduced pressure limits thermal degradation, while shade drying protects light-sensitive constituents [11,12]. A yield of about 9–10% is typical for ethanolic extraction of leafy tissue, and the greenish-brown colour indicates co-extraction of chlorophyll and phenolics. Silica gel chromatography with a chloroform-methanol gradient is a simple and widely available method for separating flavones from more polar and less polar accompanying compounds. Because leaves are agricultural residue, a route that uses inexpensive solvents and equipment is compatible with eventual use in resource-limited settings, although chloroform would need to be replaced with a less hazardous solvent in any scaled-up process.

4.8 Traditional use and ethnopharmacological relevance

Sugarcane preparations have long been used in traditional practice for urinary complaints and as a cooling diuretic. Scientific validation of such uses is uneven, and much of the published work concerns the juice rather than the leaf [11,12]. The present findings provide experimental support for a renal protective property of a leaf constituent and suggest that part of the traditional reputation of the plant may rest on flavonoids such as luteolin. They do not, however, show that traditional preparations such as juice deliver protective doses of luteolin, because the leaf and the juice differ in composition, and the luteolin content of any preparation would have to be measured before such a claim could be made.

4.9 Safety and translational considerations

No toxicity was seen in the acute oral study, which agrees with the low toxicity generally attributed to luteolin and to sugarcane preparations [11,24]. Caution is still warranted, because adverse effects of sugarcane juice on reproductive function have been reported in female rats [21], and repeat-dose and reproductive safety of any leaf-derived preparation must be established separately. From a translational perspective, aminoglycosides remain in wide use, so an inexpensive, orally available and relatively safe adjunct that limits tubular injury would be valuable. Sugarcane leaves are abundant agricultural by-products, which could make a luteolin source economically attractive. Nevertheless, luteolin has low aqueous solubility and limited oral bioavailability, and extrapolation from rats to patients must be made with caution. An important point that would need to be addressed in any translation is that the protective agent must not reduce the antibacterial efficacy of gentamicin, which was not examined here.

4.10 Limitations

Several limitations should be acknowledged. First, identity of the isolate was supported by TLC, melting point, UV and IR data, and purity was not confirmed by HPLC, nuclear magnetic resonance or mass spectrometry, so the test material is best described as a luteolin-rich fraction and minor co-eluting constituents may have contributed to activity. Second, only a single species was studied, over eight days, using concurrent (preventive) rather than post-injury treatment, so effects on established injury are unknown. Third, the mechanism was inferred mainly from MDA and was not tested by measuring antioxidant enzymes, glutathione, Nrf2 signalling, cytokines or apoptotic markers. Fourth, no group receiving luteolin alone was included, so intrinsic effects on renal function cannot be excluded, although the acute toxicity study is reassuring. Fifth, groups of six animals provide adequate power for large effects such as these but limit detection of smaller differences, and data from rats of either sex were pooled. Sixth, serum creatinine and BUN are late and relatively insensitive markers, and newer indicators of tubular injury such as kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin were not measured, although the histological findings provide direct structural confirmation. Finally, gentamicin plasma or tissue levels were not measured, so an interaction that reduced renal exposure to the antibiotic cannot be ruled out.

4.11 Future directions

Future work should confirm the structure and purity of the isolate by HPLC, NMR and mass spectrometry, and quantify luteolin in leaf material from different cultivars and seasons. Mechanistic studies should measure superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, Nrf2 and heme oxygenase-1, inflammatory cytokines such as TNF- α and IL-6, and markers of apoptosis. Longer treatment, therapeutic (post-injury) dosing, other nephrotoxins, and formulation strategies to improve bioavailability should be examined, along with confirmation that antibacterial efficacy of gentamicin is preserved.

5 Conclusion

A luteolin-rich fraction isolated from the leaves of *Saccharum officinarum* L. was safe in an acute oral toxicity study and protected rats against gentamicin-induced nephrotoxicity in a dose-dependent manner. The high dose lowered serum creatinine, BUN, uric acid and kidney MDA, normalised relative kidney weight, preserved body weight and tubular architecture, and gave about 80% protection, comparable to a standard antioxidant. The concordance of biochemical, gravimetric and histological

evidence, and the strong effect on MDA, indicate that protection is most probably mediated by suppression of oxidative injury. These findings support the traditional use of sugarcane in urinary disorders and identify luteolin from an abundant agricultural by-product as a promising nephroprotective candidate, pending confirmation of purity, mechanism and long-term safety.

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