Received: 28 April 2018 | Revised: 16 July 2018 | Accepted: 17 July 2018

DOI: 10.1002/ptr.6176

RESEARCH ARTICLE

WILEY

Oral administration of powdered dried rhizomes of *Curcuma longa* L. (turmeric, Zingiberaceae) is effective in the treatment of doxorubicin-induced kidney injury in rats

Enzo R. Russo[1] | Inalda Facincani[1] | Katia C. Nakazato[1] | Terezila M. Coimbra[1] | Eduardo J. Crevelin[2] | Ana Maria S. Pereira[3] | Fabio Carmona[1]

[1] Ribeirão Preto Medical School, University of São Paulo (USP), Ribeirão Preto, São Paulo, Brazil

[2] Department of Chemistry, Faculty of Philosophy, Sciences and Letters at Ribeirao Preto, University of São Paulo (USP), Ribeirão Preto, São Paulo, Brazil

[3] University of Ribeirão Preto (UNAERP), Ribeirão Preto, São Paulo, Brazil

Correspondence
Fabio Carmona, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Avenida dos Bandeirantes S/N, Monte Alegre, Ribeirão Preto, SP 14049-900, Brazil.
Email: carmona@fmrp.usp.br

Curcumin is a polyphenol present in the rhizomes of the species *Curcuma longa* L. ("turmeric," Zingiberaceae), which has been used for centuries as an anti-inflammatory. We aimed to evaluate the anti-inflammatory effects of *C. longa* in renal injury induced by doxorubicin (DOX, 3.5 $mg.kg^{-1}$ IV). We studied four groups of Wistar rats: two groups with DOX-induced kidney injury, one fed with standard food and another with standard food mixed with *C. longa* (5 $mg.g^{-1}$). Two other control groups without kidney injury were fed with the same foods. We measured albuminuria, body weight, and food intake every 2 weeks. After 8 weeks, treatment with *C. longa* did not change albuminuria, but it significantly attenuated the excretion of urinary inflammatory markers monocyte chemoattractant protein-1 (MCP-1) and transforming growth factor-β (TGF-β) and significantly attenuated immunostaining for desmin, vimentin, and ED-1^+ cells in renal tissues of rats with DOX-induced kidney injury. In addition, treatment with *C. longa* resulted in significantly lower glomerular and tubule interstitial injury scores, compared with that in the DOX-STD group. In conclusion, administration of powdered rhizomes of *C. longa* for 8 weeks to rats with DOX-induced kidney injury did not reduce albuminuria but led to a significant decrease in urinary inflammatory markers MCP-1 and TGF-β and decreased histopathological alterations and immunostaining for desmin, vimentin, and ED-1^+ cells kidneys tissues.

KEYWORDS

Curcuma longa, doxorubicin, herbal medicine, inflammation, proteinuria, Zingiberaceae

1 | INTRODUCTION

Nephrotic syndrome (NS) is a set of signs and symptoms characterized by proteinuria, hypoalbuminemia, and edema. Focal segmental glomerulosclerosis (FSGS) is the main cause of NS in adults, with a prevalence of around 40% (D'Agati, Kaskel, & Falk, 2011), whereas minimal change disease is the leading cause of NS in children, accounting for only 10–25% of cases in adults (Hogan & Radhakrishnan, 2013). The main therapy for adults and children is long-term use of corticosteroids. In patients who have contraindication to corticosteroids or in those who do not respond, other immunosuppressive agents have been used, including cyclophosphamide, cyclosporin, mycophenolate mofetil, and tacrolimus.

The cause of NS remains unknown, but the pathogenesis of human idiopathic NS can result from immune dysregulation, systemic circulating factors, or inherited structural abnormalities of the podocytes with loss or altered functions of these cells, resulting in massive proteinuria (Noone, Iijima, & Parekh, 2018).

Albuminuria leads to expression of chemokines in the proximal tubule through a mechanism dependent on nuclear factor κ-B (NF-κB)

activation (Donadelli et al., 2000). Moreover, a persistent, chronic, low-grade kidney inflammation often leads to chronic kidney disease (CKD; Akchurin & Kaskel, 2015). Most cases will respond to treatment with corticosteroids because they inhibit NF-κB activation (Wenderfer, 2012). However, long-term use of corticosteroids can cause undesirable side effects, such as growth retardation in children. Therefore, new, safer drugs are needed to treat NS.

One of the most promising anti-inflammatory and immunosuppressive molecules is curcumin (Amirghofran, 2012; Jagetia & Aggarwal, 2007), a polyphenol present in the rhizomes of *Curcuma longa* L. ("turmeric," Zingiberaceae), which has been used for centuries as an anti-inflammatory medicine in Asia. Turmeric and curcumin have a myriad of biological effects, including antimicrobial (Vaughn et al., 2017), anticancer (Hamzehzadeh, Atkin, Majeed, Butler, & Sahebkar, 2018), antidepressant (Al-Karawi, Al Mamoori, & Tayyar, 2016), anti-inflammatory, and analgesic in osteoartritis (Dragos et al., 2017), especially on the circulatory system (Saeidinia et al., 2018; Sukardi et al., 2015; Wongcharoen et al., 2012) and metabolic syndrome (Atkin, Katsiki, Derosa, Maffioli, & Sahebkar, 2017).

We herein tested the hypothesis that, in an animal model of doxorubicin (DOX)-induced kidney injury, as a surrogate model for NS, the use of curcumin-rich powdered rhizomes of *C. longa*, mixed in food, results in reduction of albuminuria and renal inflammation, when compared with standard food.

2 | MATERIALS AND METHODS

Male, 6- to 8-week-old Wistar rats (220–290 g of body weight) were used. They were kept in cages containing three to four rats fed with standard food and offered water *ad libitum*. For the wake–sleep cycle, the rats were exposed to 12 hr of light and 12 hr of dark, at a temperature of 22°C. Every 2 weeks, the rats were placed in individual metabolic cages for body weight, food intake, and urinary volume measurements. Urine samples were collected for measurement of albumin and creatinine concentrations. The protocol was conducted in accordance with the ethical principles of the National Council for Animal Experimentation Control (CONCEA), the EU Directive 2010/63/EU for animal experiments, and was approved by the local animal ethics committee (#057/2014).

2.1 | Preparation of *Curcuma longa*

Rhizomes of *C. longa*, cultivated in the region of Jardinopolis, São Paulo, Brazil, were collected, washed, sliced, and dried for 7 days in a circulating air oven at 45°C. This species is not protected by law in Brazil. The dried material was powdered in a knife mill up to 42-mesh particle size. Curcumin and desmethoxycurcumin standards were obtained from the rhizomes following the methods described elsewhere (Jayaprakasha, Rao, & Sakariah, 2002) and validated by spectroscopic methods. Briefly, curcuminoids were quantified by high-performance liquid chromatography, obtaining a concentration of 45.31 mg/g (*w*/*w*) of curcumin and 6.94 mg/g (*w*/*w*) of desmethoxycurcumin in the powder. For more details, see the Supporting Information provided.

2.2 | Preparation of food and choice of dose

According to the World Health Organization, the dose of powdered plant material of *C. longa* for adults is 1.5 to 3.0 g daily (WHO, 1999), corresponding, in a 70-kg adult, to 20 to 40 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$. We chose to use 40 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$. Following the FDA guidelines on dose extrapolation (United States, 2005), this dose corresponds to 250 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ in rats.

We used standard food (Nuvilab®, Curitiba, Brazil), which was crushed in a mill (DPM-4, Nogueira, Itapira, Brazil) with Sieve number 2 and mixed with the powdered *C. longa* in a proportion of 5 mg per 1 g of food. This proportion was chosen to provide a dose of 250 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ of *C. longa*, given an average food consumption of 50 $g{\cdot}kg^{-1}{\cdot}day^{-1}$ for an adult rat (Carvalho et al., 2009).

This mixture was mixed (mixer MA 206, Marconi, Piracicaba, Brazil) for 10 min and then pelletized (Rosim 0337, Rosim, Boa Esperança do Sul, Brazil) back.

2.3 | Induction of kidney injury

We used a model of DOX-induced kidney injury as a surrogate model for NS (Bertani et al., 1982; Simic, Tabatabaeifar, & Schaefer, 2013). DOX (Fauldoxo®, Libbs, Embu, Brazil), at 3.5 $mg.kg^{-1}$, was injected IV through the tail dorsal vein on Day 0 (D0). According to Lee and Harris (2011), the dose of DOX to induce nephropathy in rats ranges between 1.5 and 7.5 $mg.kg^{-1}$ (Lee & Harris, 2011). In our study, we selected a dose of 3.5 $mg.kg^{-1}$, which was previously used by our group (Galli, Volpini, Costa, da Silva, & Coimbra, 2001).

The animals were then split into four groups, with 9–10 rats each, as follows: SAL-STD—the rats were treated with normal saline (SAL) and received standard food (STD) during the treatment period (n = 9); SAL-CUR—the rats were treated with SAL and received standard food mixed with *C. longa* (CUR; n = 10); DOX-STD—the rats were treated with DOX and received standard food (STD; n = 10); and DOX-CUR—the rats were treated with DOX and received standard food mixed with *C. longa* (CUR; n = 10).

On Day 56, the rats were anesthetized with sodium thiopental (40 $mg.kg^{-1}$, i.p.), and after completely sedated, blood and urine samples were collected. A catheter was surgically inserted in the inferior vena cava for blood collection, and another catheter was surgically inserted in the bladder for urine collection. The animals were then sacrificed, and the left kidney was harvested, sectioned, and fixed in methanol-Carnoy (methacarn) solution for 24 hr and stored in 70% ethanol. Histological sections were made for optical microscopy and immunolabelling for desmin, vimentin, and ED-1^+ cells.

2.4 | Determination of serum and urinary tests

Urine and serum samples were kept frozen at −70°C until processed. Urine samples were preserved by the addition of sodium azide. Urinary albumin and creatinine measurements were made by immunoturbidimetric and kinetic methods, respectively (2300 Plus Wiener Metrolab, Wiener Laboratorios, Rosario, Argentina; Wiener Microalbumina Turbitest AA; Wiener Creatinina Cinetica AA). The albumin/creatinine ratio was used for normalization of albuminuria. In samples collected on Day 56, serum and urinary osmolality were

estimated by the steam reduction pressure point (Wescor Vapro Pressure Osmometer 5600, Wescor, Puteaux, France). Serum creatinine was measured by kinetic method (2300 Plus Wiener Metrolab, Wiener Laboratorios, Rosario, Argentina; Wiener Cinetic Creatinine AA). Urine samples for the determination of monocyte chemoattractant protein-1 (MCP-1) and transforming growth factor-β (TGF-β) were treated immediately with 1 mM of phenyl-methane-sulfonyl-fluoride. Quantification was done by enzyme-linked immunosorbent assay, using commercial kits Promega and Pierce, respectively (Promega Corporation, Madison, MO, USA, and Pierce Biotechnology, Rockford, IL, USA). The values of TGF-β and MCP-1 were expressed in picogrammes of TGF-β or MCP-1 per milligramme of creatinine (pg/mg).

2.5 | Light microscopy

The histological sections (3 μm) were stained with Masson's trichrome. Glomerular lesions were evaluated by scores that reflected the extent of the injury (0, 0–5%; 1, 5–25%; 2, 25–50%; 3, 50–75%; or 4, >75%). Interstitial injuries of the renal cortex were also evaluated by scores (Shih, Hines, & Neilson, 1988; 0, *normal*; 0.5, *discrete focal changes*; 1, *<10% of the cortex*; 2, *10–25% of the cortex*; 3, *25–75% of the cortex*; or 4, *>75% of the cortex*). These scores were assessed in 50 glomeruli and 20 optical fields (0.245 mm^2/field) of each animal. Mean scores of glomerular and interstitial lesions were calculated for each animal.

2.6 | Immunohistochemistry

The histological sections (3 μm) were dewaxed and subjected to immunolabelling. The material was incubated for 60 min at room temperature with the following primary antibodies: (a) antidesmin monoclonal (Dako A/S, Denmark, 1/100); (b) antivimentin monoclonal (Dako A/S, Denmark, 1/500); and (c) anti-ED-1 monoclonal (Serotec, USA, 1/1000).

The sections were then washed with phosphate-buffered SAL and incubated with biotinylated secondary antibodies anti-immunoglobulin G. The product of the reaction was detected with avidin-biotin-peroxidase system (Vector Laboratories, USA), and color was developed by adding 3,3-diaminobenzidine (Sigma Chemical Company, USA) in the presence of hydrogen peroxide. Nonspecific links have been blocked by primary and secondary antibody dilutions with phosphate-buffered SAL solution containing bovine albumin (1%). The counterstain was performed with methylgreen. The mean number of ED-1^+ cells per field and per glomerulus for each animal was calculated. For all the immunostainings above, at least 40 fields (0.245 mm^2) and 50 glomeruli were analyzed.

2.7 | Statistical analysis

Comparisons between groups were done with one-way (single time point) or two-way (repeated measures) analysis of variance, with Tukey–Kramer test for multiple comparisons. Some data were log-transformed before statistical analysis because of non-normal distributions. Values of $p < 0.05$ were considered statistically significant. We used GraphPad Prism 7 software (GraphPad Software Inc., La Jolla, CA, USA).

3 | RESULTS

Weight gain was lower in DOX-treated animals by approximately 100 g, although this difference was not statistically significant. Food intake did not differ significantly between groups. Drug intake within food was around 400 $mg{\cdot}kg^{-1}{\cdot}day^{-1}$ in both groups treated with *C. longa* (nonsignificant difference, Table 1).

All groups had normal levels of serum creatinine and serum and urinary osmolality. Urinary osmolality was significantly lower in the DOX-CUR group when compared with SAL-CUR, and no other difference was found (Table 2).

The urinary albumin/creatinine ratio was significantly higher in sick animals (DOX-STD and DOX-CUR) as compared with that in nonsick animals (SAL-STD and SAL-CUR) from Day 14 on; however, treatment with *C. longa* did not affect this ratio (Figure 1).

TABLE 1 Weight gain and food and drug (powdered *Curcuma longa* rhizomes) intake

Group	Weight gain (g)	Food intake ($g.day^{-1}$)	Powdered *Curcuma longa* intake ($mg{\cdot}kg^{-1}{\cdot}day^{-1}$)
SAL-STD	367 ± 64	34.0 (32.5, 39.1)	–
SAL-CUR	359 ± 39	36.3 (33.4, 39.3)	382 (331, 431)
DOX-STD	252 ± 59	35.9 (34.9, 38.6)	–
DOX-CUR	257 ± 59	35.9 (32.5, 37.4)	415 (352, 445)

Note. DOX: doxorubicin; CUR: food plus powdered *Curcuma longa* rhizomes; SAL: normal saline; STD: standard food. Nine animals were studied in group SAL-STD, and 10 animals per group were studied in the other groups. Data are expressed as median (interquartile range) or mean ± standard deviation.

TABLE 2 Urinary creatinine and serum and urinary osmolality

Group	Urinary creatinine ($mg.dL^{-1}$)	Serum osmolality ($mmol.kg^{-1}$)	Urinary osmolality ($mmol.kg^{-1}$)
SAL-STD	0.49 (0.43, 0.54)	302 (300, 305)	1,573 (1,365, 1,785)
SAL-CUR	0.42 (0.38, 0.43)	299 (297, 301)	2,057 (1,771, 2,152)
DOX-STD	0.40 (0.34, 0.43)	298 (297, 305)	1,513 (1,184, 1,946)
DOX-CUR	0.36 (0.26, 0.51)	298 (296, 300)	1,193 (912, 1,646)

Note. DOX: doxorubicin; CUR: food with powdered *Curcuma longa* rhizomes; SAL: normal saline; STD: standard food. Nine animals were studied in group SAL-STD, and 10 animals per group were studied in the other groups. The data were expressed as median (interquartile range).

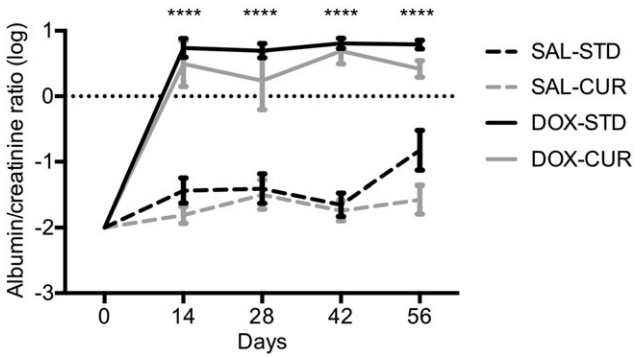

FIGURE 1 Log-transformed albumin/creatinine ratio (mean and standard error of the mean). Nine animals were studied in group SAL-STD, and 10 animals per group were studied in the other groups. DOX: doxorubicin; CUR: food with powdered *Curcuma longa* rhizomes; SAL: normal saline; STD: standard food; ****: $p < 0.0001$ for all comparisons between SAL and DOX groups

3.1 | Kidney inflammation and injury

Urinary MCP-1/creatinine and TGF-β/creatinine ratios were both significantly higher in sick, nontreated animals (DOX-STD) when compared with those in nonsick animals (SAL-STD and SAL-CUR groups). Treatment with *C. longa* (DOX-CUR group) led to a significant reduction on both urinary MCP-1/creatinine and TGF-β/creatinine ratios (Figure 2).

Light microscopy revealed, in sick animals (DOX-STD and DOX-CUR), glomerulosclerosis, tubular lumen dilation, flattening of the tubular cells with loss of brush border, tubular atrophy, and interstitial fibrosis. Glomerular and tubule interstitial injury scores were significantly higher in sick animals (DOX-STD and DOX-CUR), and treatment with *C. longa* (DOX-CUR) resulted in significantly lower scores, compared with that in DOX-STD group (Figure 3).

Results obtained from immunostaining for desmin, vimentin, and ED-1^+ cells are shown in Figures 4, 5, and 6, respectively. Induction of kidney injury (DOX-STD and DOX-CUR) resulted in significantly higher staining scores for desmin, vimentin, and ED-1^+ cells, both in glomeruli and in interstitial tissue. Moreover, treatment with *C. longa* (DOX-CUR) resulted in significantly lower scores for all stainings except for ED-1^+ cells in glomeruli.

4 | DISCUSSION

We herein confirmed our hypothesis that oral administration of powdered rhizomes of *C. longa* to animals with DOX-induced kidney injury reduces tissue inflammation, demonstrated by the lower expression of urinary inflammatory markers MCP-1 and TGF-β and by attenuated immunostaining for desmin, vimentin, and ED-1^+ cells in kidney tissue. However, albuminuria was not reduced.

MCP-1 recruits monocytes and macrophages to tissues, whereas TGF-β promotes tissue fibrosis. Urinary concentrations of MCP-1 and TGF-β correlate significantly with tissue expression of the same proteins and, therefore, can be used as surrogate markers for renal tissue inflammation (De Muro et al., 2004; M. J. Kim & Tam, 2011). Both markers were reduced with treatment with *C. longa*, suggesting a milder inflammation in the renal tissues of the group treated with *C. longa*.

Desmin is a component of the cytoskeleton, considered as a sensitive marker of injury in foot processes, whereas vimentin expression is suggestive of recent or ongoing tubular injury. Both were reduced by treatment with *C. longa*, suggesting less glomerular and interstitial injury in the kidneys of the group treated with *C. longa*.

The monoclonal antibody anti-ED-1 marks macrophages and monocytes, which are present in inflamed tissues. In our study, we observed a significantly smaller number of ED-1^+ cells in the tubule-interstitial compartment of the animals treated with *C. longa*. The same did not occur for ED-1^+ cells in the glomerular compartment. This finding suggests that there was a more intense inflammation in the tubular compartment than in the glomerular compartment. The lower urinary osmolality that we observed in the group of sick, treated animals (DOX-CUR) suggests that there was a loss of tubular concentration function, which takes place in the tubule-interstitial compartment.

Our experiment showed that animals with kidney injury treated with *C. longa* had lower markers of kidney inflammation and injury. These observations were confirmed by light microscopy, in which we observed attenuation of glomerulosclerosis, tubular atrophy, and interstitial fibrosis, when compared with sick, untreated animals (DOX-STD).

In the groups with DOX-induced kidney injury, albuminuria remained high during the 8 weeks of the experiment. The choice of 8 weeks for the experiment was made to evaluate the animals during the period of absent or mild tissue injury induced by DOX. Our model worked very alike that described in the literature (Bertani et al., 1982; Bertani, Cutillo, Zoja, Broggini, & Remuzzi, 1986; Galli et al., 2001), with albuminuria appearing in the first weeks. The kidney injury model with DOX causes increased glomerular capillary permeability and tubular atrophy (Noone et al., 2018), leading to massive proteinuria, hypoalbuminemia, hyperlipidemia, and almost no histological lesion in the first 8 weeks. That is why this model is often used as a surrogate

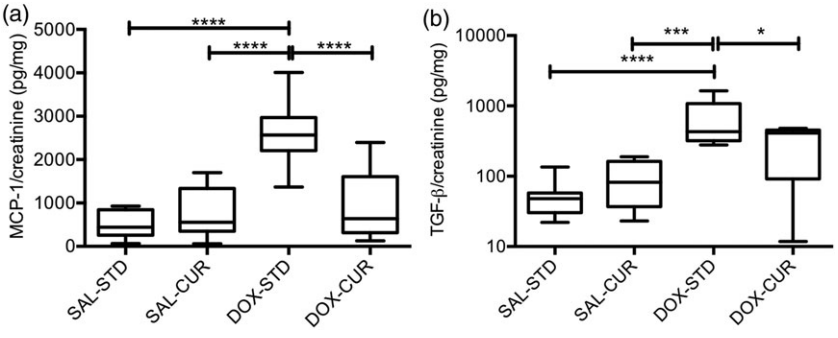

FIGURE 2 (a) MCP-1/creatinine and (b) TGF-β/creatinine ratios at the 56th day. Nine animals were studied in group SAL-STD, and 10 animals per group were studied in the other groups. MCP-1: monocyte chemoattractant protein-1; TGF-β: transforming growth factor-β; DOX: doxorubicin; CUR: food with powdered *Curcuma longa* rhizomes; SAL: normal saline; STD: standard food; *: $p < 0.05$; ***: $p < 0.001$; ****: $p < 0.0001$

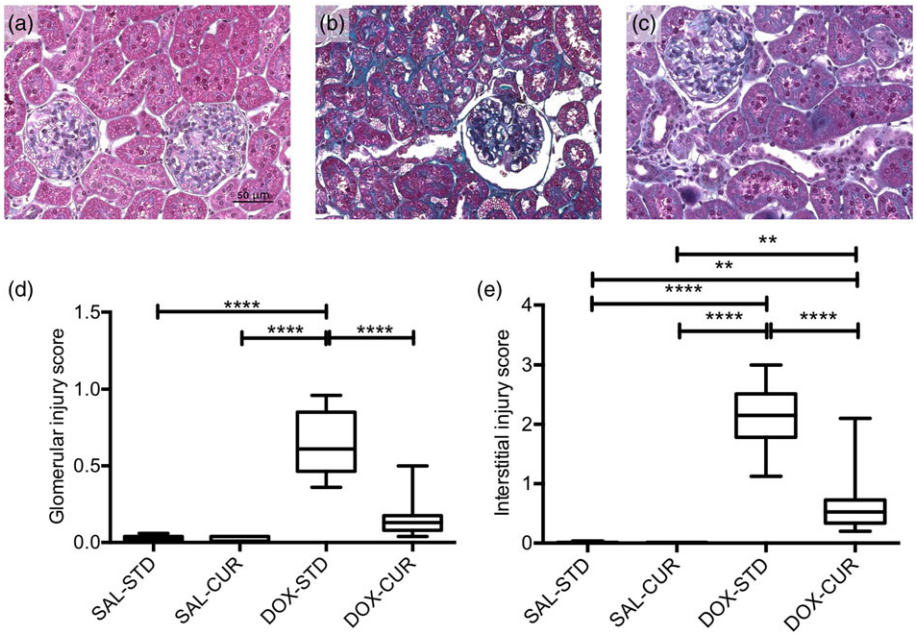

FIGURE 3 Histological sections of renal cortex stained with Masson's trichrome (bar indicates 50 µm) from the following groups: (a) control (SAL-STD), (b) animal with kidney injury fed with standard food (DOX-STD), and (c) animal with kidney injury fed with food containing powdered *Curcuma longa* rhizomes (DOX-CUR); and graph comparisons of (d) glomerular injury score and (e) interstitial injury score between groups. Nine animals were studied in group SAL-STD, and 10 animals per group were studied in the other groups. DOX: doxorubicin; CUR: food with powdered *C. longa* rhizomes; SAL: normal saline; STD: standard food; **: $p < 0.01$; ****: $p < 0.0001$ [Colour figure can be viewed at wileyonlinelibrary.com]

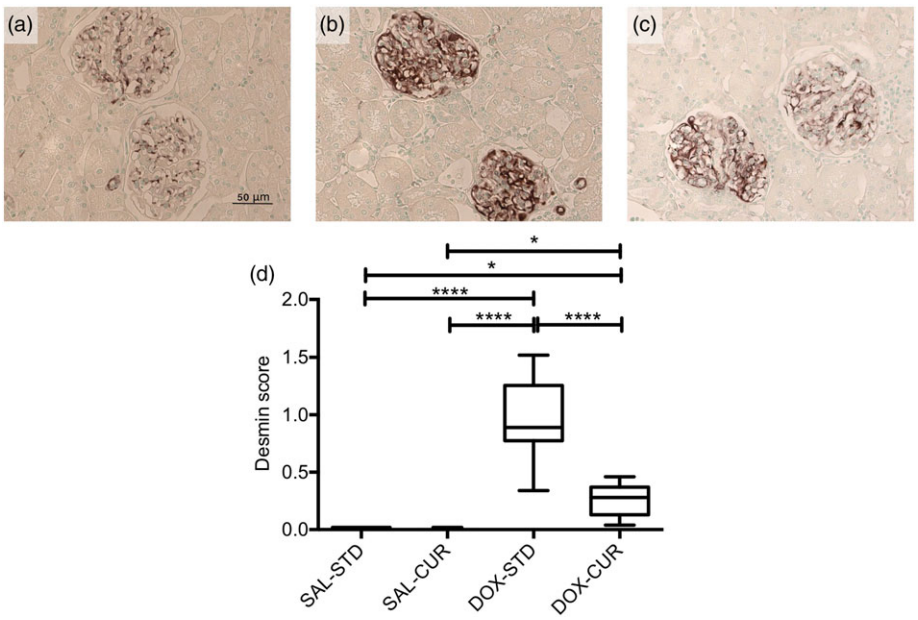

FIGURE 4 Histological sections of renal cortex with immunolabelling for desmin (bar indicates 50 µm) from the following groups: (a) control (SAL-STD), (b) animal with kidney injury fed with standard food (DOX-STD), and (c) animal with kidney injury fed with food containing powdered *Curcuma longa* rhizomes (DOX-CUR); and graph comparisons of (d) desmin score. Nine animals were studied in group SAL-STD, and 10 animals per group were studied in the other groups. DOX: doxorubicin; CUR: food with powdered *C. longa* rhizomes; SAL: normal saline; STD: standard food; *: $p < 0.05$; ****: $p < 0.0001$ [Colour figure can be viewed at wileyonlinelibrary.com]

model for NS. However, after 8 weeks, more extensive lesions appear in the kidneys, evolving to FSGS and CKD (Okuda et al., 1986). In addition, the glomerulonephritis that causes albuminuria in the model with DOX is secondary to drug nephrotoxicity, a different mechanism of proteinuria from that seen in humans with NS. These differences could explain why we did not observe any reduction in albuminuria. Regarding toxicity, the addition of *C. longa* to the food resulted in no observable adverse effect to the animals.

Curcumin, the main active constituent of *C. longa*, besides a myriad of pharmacological properties (Jurenka, 2009; Nelson et al., 2017),

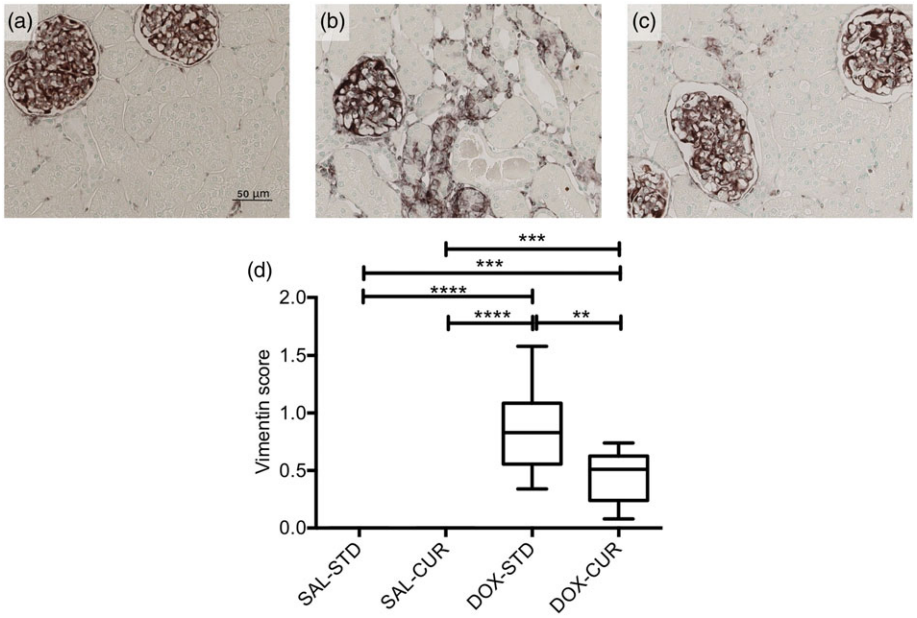

FIGURE 5 Histological sections of renal cortex with immunolabelling for vimentin (bar indicates 50 µm) from the following groups: (a) control (SAL-STD), (b) animal with kidney injury fed with standard food (DOX-STD), and (c) animal with kidney injury fed with food containing powdered *Curcuma longa* rhizomes (DOX-CUR); and graph comparisons of (d) vimentin score. Nine animals were studied in group SAL-STD, and 10 animals per group were studied in the other groups. DOX: doxorubicin; CUR: food with powdered *C. longa* rhizomes; SAL: normal saline; STD: standard food; *: $p < 0.05$; ***: $p < 0.001$; ****: $p < 0.0001$ [Colour figure can be viewed at wileyonlinelibrary.com]

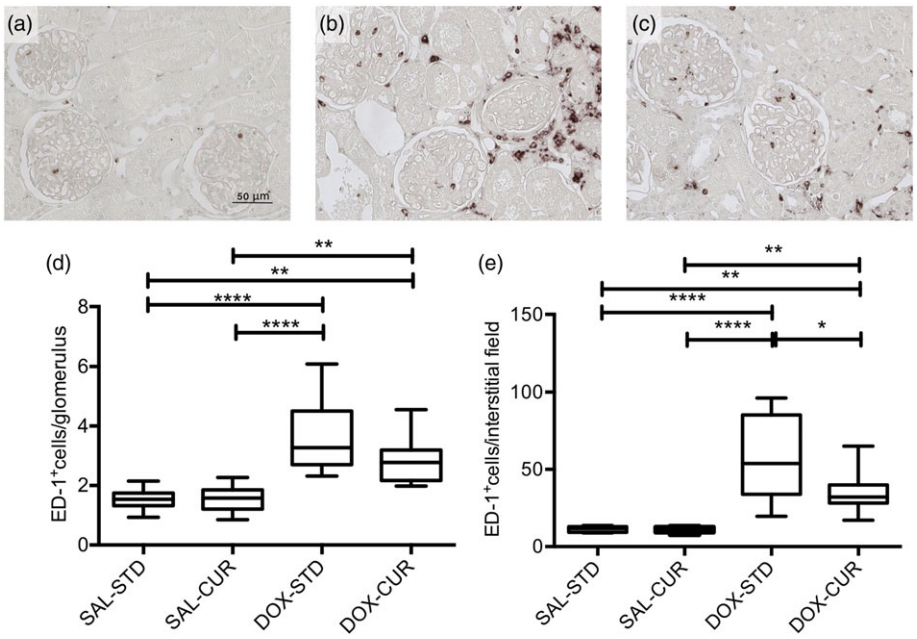

FIGURE 6 Histological sections of renal cortex with immunolabelling for ED-1^+ cells (bar indicates 50 µm) from the following groups: (a) control (SAL-STD), (b) animal with kidney injury fed with standard food (DOX-STD), and (c) animal with kidney injury fed with food containing powdered *Curcuma longa* rhizomes (DOX-CUR); and graph comparisons of ED-1^+ cells in (d) glomeruli and (e) interstitial tissue. Nine animals were studied in group SAL-STD, and 10 animals per group were studied in the other groups. DOX: doxorubicin; CUR: food with powdered *C. longa* rhizomes; SAL: normal saline; STD: standard food; *: $p < 0.05$; **: $p < 0.01$; ****: $p < 0.0001$ [Colour figure can be viewed at wileyonlinelibrary.com]

including anti-inflammatory and antioxidant, has also been investigated for its potential to prevent and treat renal diseases. In rat renal fibroblasts, curcumin blocked TGF-β profibrotic actions through down-regulation of TGF-β receptor type II and through partial inhibition of c-jun activity (Gaedeke, Noble, & Border, 2004). Curcumin also induced expression of mesangial cell heme-oxygenase 1 and up-regulated glomerular heme-oxygenase 1 expression in nephritic animals in vivo (anti-Thy 1 glomerulonephritis model), leading to a significant, dose-dependent reduction of markers of fibrosis and proteinuria (Gaedeke, Noble, & Border, 2005).

C. longa is already used in humans as an anti-inflammatory in various situations, such as in the treatment of osteoarthritis

(Kuptniratsaikul et al., 2014), atherogenic risk reduction in patients with Type 2 diabetes mellitus (Chuengsamarn, Rattanamongkolgul, Phonrat, Tungtrongchitr, & Jirawatnotai, 2014), and attenuation of myocardial injury after cardiac surgery (Wongcharoen et al., 2012). In addition, the plant exhibits protective effects in animal models of myocardial (Tanwar, Sachdeva, Golechha, Kumari, & Arya, 2010; Yang, Wu, Li, & You, 2013), pulmonary (K. Liu et al., 2012; Sakurai et al., 2013), and renal injury (Mohamad et al., 2009; Sharma, Kulkarni, & Chopra, 2006). In rats, *C. longa* attenuated nephrotoxicity of gentamicin and cisplatin (Elgazar & AboRaya, 2013; Pathak, Rajurkar, Tarekh, & Badgire, 2013) and also protected the heart, liver, and kidneys from DOX-induced toxicity (Mohamad et al., 2009). Curcumin also protected the kidneys against cadmium-induced nephrotoxicity (K. S. Kim et al., 2018), ischemia–reperfusion injury (F. Liu et al., 2017), and, in the form of nanoparticles, against rhabdomyolysis-induced kidney injury (Chen et al., 2018). In these studies, however, *C. longa* was used in different ways. In one study, a *C. longa* extract was administered 10 days prior to the injection of DOX, in a preventive way (Mohamad et al., 2009); in another study, an oral suspension of curcumin was used in the reduction of oxidative stress and renal dysfunction in diabetic rats (Sharma et al., 2006); and in yet another study, a curcumin solution was injected previously to lipopolysaccharide-induced injury, showing reduction of renal MCP-1 e NF-κB activation (Zhong, Chen, Han, Jin, & Wang, 2011).

This is the first study that, to the best of our knowledge, evaluated the therapeutic efficacy of *C. longa*, administered after DOX-induction of injury and in the form of powdered dried rhizomes. This means that we investigated the therapeutic, not the prophylactic, effect. In addition, we used powdered plant material; therefore, producing this medicine does not require expensive or complex technology. In fact, there is preclinical evidence that the effect of *C. longa* is superior to that of isolated curcumin (Martin, Aiyer, Malik, & Li, 2012). This may be explained because isolated curcumin is poorly soluble in water and has limited bioavailability (Jurenka, 2009). Curcumin bioavailability can be substantially increased by concomitant administration of piperine (from black pepper, *Piper nigrum* L.), but the best bioavailability of curcumin has been obtained by mixing it with compounds initially present in turmeric roots that are removed by the puritification process, especially Ar-turmerone (Antony et al., 2008). This enhanced bioavailability is a result of the inherent synergism between the natural compounds present in the rhizomes (Kiefer, 2007). Another explanation for a superior effect of *C. longa* is that other compounds, such as turmerone, can also have therapeutic effects.

In patients with diabetic nephropathy, short-term administration of *C. longa* attenuated proteinuria and serum levels of TGF-β and interleukin-8 (Khajehdehi et al., 2011). In patients with chronic renal disease, administration of curcumin was well tolerated and reduced interleukin-6 levels (Moreillon et al., 2013).

Our results encourage us to conduct a clinical trial to investigate the effectiveness of *C. longa* to treat idiopathic NS and other forms of inflammatory kidney disease in humans. We can speculate that *C. longa* can be also useful in other syndromes with proteinuria, because reducing kidney inflammation could lead to a slower progression to CKD. Of course, more studies are needed to confirm these speculations.

In conclusion, the administration of powdered dried rhizomes of *C. longa*, to rats with DOX-induced renal injury, for 8 weeks, resulted in reduction of urinary markers of renal inflammation MCP-1 and TGF-β, and lower tissue expression of markers of renal injury desmin and vimentin, and a smaller number of ED-1^+ cells (macrophages and monocytes) in tubule-interstitial compartment, as well as less renal tissue damage in light microscopy, suggesting an anti-inflammatory effect in the kidneys.

ACKNOWLEDGMENT

We thank to the Laboratory of Renal Physiology, Ribeirão Preto Medical School, University of São Paulo, for providing materials and technical assistance to carry out this work.

ORCID

Enzo R. Russo http://orcid.org/0000-0003-0282-6204
Ana Maria S. Pereira http://orcid.org/0000-0002-3478-4718
Fabio Carmona http://orcid.org/0000-0001-5743-0325

REFERENCES

Akchurin, O. M., & Kaskel, F. (2015). Update on inflammation in chronic kidney disease. *Blood Purification*, *39*(1–3), 84–92.

Al-Karawi, D., Al Mamoori, D. A., & Tayyar, Y. (2016). The role of curcumin administration in patients with major depressive disorder: Mini meta-analysis of clinical trials. *Phytotherapy Research*, *30*(2), 175–183. https://doi.org/10.1002/ptr.5524

Amirghofran, Z. (2012). Herbal medicines for immunosuppression. *Iranian Journal of Allergy, Asthma, and Immunology*, *11*(2), 111–119. https://doi.org/011.02/ijaai.111119

Antony, B., Merina, B., Iyer, V. S., Judy, N., Lennertz, K., & Joyal, S. (2008). A pilot cross-over study to evaluate human oral bioavailability of BCM-95CG (Biocurcumax), a novel bioenhanced preparation of curcumin. *Indian Journal of Pharmaceutical Sciences*, *70*(4), 445–449. https://doi.org/10.4103/0250-474X.44591

Atkin, S. L., Katsiki, N., Derosa, G., Maffioli, P., & Sahebkar, A. (2017). Curcuminoids lower plasma leptin concentrations: A meta-analysis. *Phytotherapy Research*, *31*(12), 1836–1841. https://doi.org/10.1002/ptr.5905

Bertani, T., Cutillo, F., Zoja, C., Broggini, M., & Remuzzi, G. (1986). Tubulointerstitial lesions mediate renal damage in adriamycin glomerulopathy. *Kidney International*, *30*(4), 488–496.

Bertani, T., Poggi, A., Pozzoni, R., Delaini, F., Sacchi, G., Thoua, Y., … Donati, M. B. (1982). Adriamycin-induced nephrotic syndrome in rats: Sequence of pathologic events. *Laboratory Investigation*, *46*(1), 16–23.

Carvalho, G. D. D., Masseno, A. P. B. P. B., Zanini, M. S. S., Zanini, S. F. F., Porfirio, L. C. C., Machado, J. P. P., … Macuda, H. (2009)Avaliação clínica de ratos de laboratório (Rattus novergicus linhagem Wistar): parâmetros sanitários, biológicos e fisiológicos, 56 Revista Ceres §. Revista Ceres.

Chen, X., Sun, J., Li, H., Wang, H., Lin, Y., Hu, Y., & Zheng, D. (2018). Curcumin-loaded nanoparticles protect against rhabdomyolysis-induced acute kidney injury. *Cellular Physiology and Biochemistry*, *43*(5), 2143–2154. https://doi.org/10.1159/000484233

Chuengsamarn, S., Rattanamongkolgul, S., Phonrat, B., Tungtrongchitr, R., & Jirawatnotai, S. (2014). Reduction of atherogenic risk in patients with type 2 diabetes by curcuminoid extract: A randomized controlled trial. *The Journal of Nutritional Biochemistry*, *25*, 144–150. https://doi.org/10.1016/j.jnutbio.2013.09.013

D'Agati, V. D., Kaskel, F. J., & Falk, R. J. (2011). Focal segmental glomerulosclerosis. *The New England Journal of Medicine*, *365*(25), 2398–2411. https://doi.org/10.1056/NEJMra1106556

De Muro, P., Faedda, R., Fresu, P., Masala, A., Cigni, A., Concas, G., … Cherchi, G. M. (2004). Urinary transforming growth factor-beta 1 in various types of nephropathy. *Pharmacological Research*, *49*(3), 293–298. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14726227

Donadelli, R., Abbate, M., Zanchi, C., Corna, D., Tomasoni, S., Benigni, A., … Zoja, C. (2000). Protein traffic activates NF-kB gene signaling and promotes MCP-1-dependent interstitial inflammation. *American Journal of Kidney Diseases*, *36*(6), 1226–1241. https://doi.org/10.1053/ajkd.2000.19838

Dragos, D., Gilca, M., Gaman, L., Vlad, A., Iosif, L., Stoian, I., & Lupescu, O. (2017). Phytomedicine in joint disorders. *Nutrients*, *9*(1), 70. https://doi.org/10.3390/nu9010070

Elgazar, F. A., & AboRaya, A. O. (2013). Nephroprotective and diuretic effects of three medicinal herbs against gentamicin-induced nephrotoxicity in male rats. *Pakistan Journal of Nutrition*, *12*(8), 715–722. https://doi.org/10.3923/pjn.2013.715.722

Gaedeke, J., Noble, N. A., & Border, W. A. (2004). Curcumin blocks multiple sites of the TGF-β signaling cascade in renal cells. *Kidney International*, *66*(1), 112–120. https://doi.org/10.1111/j.1523-1755.2004.00713.x

Gaedeke, J., Noble, N. A., & Border, W. A. (2005). Curcumin blocks fibrosis in anti-Thy 1 glomerulonephritis through up-regulation of heme oxygenase 1. *Kidney International*, *68*(5), 2042–2049. https://doi.org/10.1111/j.1523-1755.2005.00658.x

Galli, L. G., Volpini, R. A., Costa, R. S., da Silva, C. G., & Coimbra, T. M. (2001). Tubular cell lesion, albuminuria, and renal albumin handling in rats treated with adriamycin. *Renal Failure*, *23*(5), 693–703. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11725916

Hamzehzadeh, L., Atkin, S. L., Majeed, M., Butler, A. E., & Sahebkar, A. (2018). The versatile role of curcumin in cancer prevention and treatment: A focus on PI3K/AKT pathway. *Journal of Cellular Physiology*, (March), *233*, 1–8. https://doi.org/10.1002/jcp.26620.

Hogan, J., & Radhakrishnan, J. (2013). The treatment of minimal change disease in adults. *Journal of the American Society of Nephrology*, *24*(5), 702–711. https://doi.org/10.1681/ASN.2012070734

Jagetia, G. C., & Aggarwal, B. B. (2007). "Spicing up" of the immune system by curcumin. *Journal of Clinical Immunology*, *27*(1), 19–35. https://doi.org/10.1007/s10875-006-9066-7

Jayaprakasha, G. K., Rao, L. J. M., & Sakariah, K. K. (2002). Improved HPLC method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. *Journal of Agricultural and Food Chemistry*, *50*(13), 3668–3672. https://doi.org/10.1021/jf025506a

Jurenka, J. S. (2009). Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: A review of preclinical and clinical research. *Alternative Medicine Review*, *14*(2), 141–153. Retrieved from http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=19594223&retmode=ref&cmd=prlinks

Khajehdehi, P., Pakfetrat, M., Javidnia, K., Azad, F., Malekmakan, L., Nasab, M. H., & Dehghanzadeh, G. (2011). Oral supplementation of turmeric attenuates proteinuria, transforming growth factor-β and interleukin-8 levels in patients with overt type 2 diabetic nephropathy: A randomized, double-blind and placebo-controlled study. *Scandinavian Journal of Urology and Nephrology*, *45*(5), 365–370. https://doi.org/10.3109/00365599.2011.585622

Kiefer, D. (2007). Novel turmeric compound delivers much more curcumin to the blood. Retrieved January 21, 2017, from http://www.lef.org/magazine/2007/10/report_curcumin/Page-01

Kim, K. S., Lim, H. J., Lim, J. S., Son, J. Y., Lee, J., Lee, B. M., … Kim, H. S. (2018). Curcumin ameliorates cadmium-induced nephrotoxicity in Sprague-Dawley rats. *Food and Chemical Toxicology*, *114*(February), 34–40. https://doi.org/10.1016/j.fct.2018.02.007

Kim, M. J., & Tam, F. W. K. (2011). Urinary monocyte chemoattractant protein-1 in renal disease. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *412*(23–24), 2022–2030. https://doi.org/10.1016/j.cca.2011.07.023

Kuptniratsaikul, V., Dajpratham, P., Taechaarpornkul, W., Buntragulpoontawee, M., Lukkanapichonchut, P., Chootip, C., … Laongpech, S. (2014). Efficacy and safety of Curcuma domestica extracts compared with ibuprofen in patients with knee osteoarthritis: A multicenter study. *Clinical Interventions in Aging*, *9*, 451–458.

Lee, V. W. S., & Harris, D. C. H. (2011). Adriamycin nephropathy: A model of focal segmental glomerulosclerosis. *Nephrology* (Carlton, Vic.), *16*(1), 30–38. https://doi.org/10.1111/j.1440-1797.2010.01383.x

Liu, F., Ni, W., Zhang, J., Wang, G., Li, F., & Ren, W. (2017). Administration of curcumin protects kidney tubules against renal ischemia-reperfusion injury (RIRI) by modulating nitric oxide (NO) signaling pathway. *Cellular Physiology and Biochemistry*, *44*(1), 401–411. https://doi.org/10.1159/000484920

Liu, K., Shen, L., Wang, J., Dong, G., Wu, H., Shao, H., & Jing, H. (2012). The preventative role of curcumin on the lung inflammatory response induced by cardiopulmonary bypass in rats. *Journal of Surgical Research*, *174*(1), 73–82. https://doi.org/10.1016/j.jss.2010.12.002

Martin, R. C. G., Aiyer, H. S., Malik, D., & Li, Y. (2012). Effect on pro-inflammatory and antioxidant genes and bioavailable distribution of whole turmeric vs curcumin: Similar root but different effects. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, *50*(2), 227–231. https://doi.org/10.1016/j.fct.2011.10.070

Mohamad, R. H., El-Bastawesy, A. M., Zekry, Z. K., Al-Mehdar, H. A., Al-said, M. G. A. M., Aly, S. S., … El-Merzabani, M. M. (2009). The role of *Curcuma longa* against doxorubicin (adriamycin)-induced toxicity in rats. *Journal of Medicinal Food*, *12*(2), 394–402. https://doi.org/10.1089/jmf.2007.0715

Moreillon, J. J., Bowden, R. G., Deike, E., Griggs, J., Wilson, R., Shelmadine, B., … Beaujean, A. (2013). The use of an anti-inflammatory supplement in patients with chronic kidney disease. *Journal of Complementary & Integrative Medicine*, *10*. https://doi.org/10.1515/jcim-2012-0011

Nelson, K. M., Dahlin, J. L., Bisson, J., Graham, J., Pauli, G. F., & Walters, M. A. (2017). The essential medicinal chemistry of curcumin. *Journal of Medicinal Chemistry*, *60*(5):1620-1637. acs.jmedchem.6b00975. https://doi.org/10.1021/acs.jmedchem.6b00975

Noone, D. G., Iijima, K., & Parekh, R. (2018). Idiopathic nephrotic syndrome in children. *The Lancet*, *392*(10141), 61–74. https://doi.org/10.1016/S0140-6736(18)30536-1

Okuda, S., Oh, Y., Tsuruda, H., Onoyama, K., Fujimi, S., & Fujishima, M. (1986). Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease. *Kidney International*, *29*(2), 502–510.

Pathak, N., Rajurkar, S., Tarekh, S., & Badgire, V. (2013). Nephroprotective effects of carvedilol and *Curcuma longa* against cisplatin-induced nephrotoxicity in rats. *Asian Journal of Medical Sciences*, *5*(2), 91–98. https://doi.org/10.3126/ajms.v5i2.5483

Saeidinia, A., Keihanian, F., Butler, A. E., Bagheri, R. K., Atkin, S. L., & Sahebkar, A. (2018). Curcumin in heart failure: A choice for complementary therapy? *Pharmacological Research*, *131*(March, 112–119. https://doi.org/10.1016/j.phrs.2018.03.009

Sakurai, R., Villarreal, P., Husain, S., Liu, J., Sakurai, T., Tou, E., … Rehan, V. K. (2013). Curcumin protects the developing lung against long-term hyperoxic injury. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, *305*(4), L301–L311. https://doi.org/10.1152/ajplung.00082.2013

Sharma, S., Kulkarni, S. K., & Chopra, K. (2006). Curcumin, the active principle of turmeric (*Curcuma longa*), ameliorates diabetic nephropathy in rats. *Clinical and Experimental Pharmacology & Physiology*, *33*(10), 940–945. https://doi.org/10.1111/j.1440-1681.2006.04468.x

Shih, W., Hines, W. H., & Neilson, E. G. (1988). Effects of cyclosporin A on the development of immune-mediated interstitial nephritis. *Kidney International*, *33*(6), 1113–1118. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3261370

Simic, I., Tabatabaeifar, M., & Schaefer, F. (2013). Animal models of nephrotic syndrome. *Pediatric Nephrology*, *28*(11), 2079–2088. https://doi.org/10.1007/s00467-012-2376-5

Sukardi, R., Sastroasmoro, S., Siregar, N. C., Djer, M. M., Suyatna, F. D., Sadikin, M., … Witarto, A. B. (2015). The role of curcumin as an inhibitor of oxidative stress caused by ischaemia re-perfusion injury in tetralogy of Fallot patients undergoing corrective surgery. *Cardiology in the Young*, *26*, 1–8. https://doi.org/10.1017/S1047951115000360.

Tanwar, V., Sachdeva, J., Golechha, M., Kumari, S., & Arya, D. S. (2010). Curcumin protects rat myocardium against isoproterenol-induced ischemic injury: attenuation of ventricular dysfunction through increased expression of Hsp27 along with strengthening antioxidant defense system. *Journal of Cardiovascular Pharmacology*, *55*(4), 377–384. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20125031

United States. (2005). FDA guidance for industry—Estimating the maximum safe dose in initial clinical trials for therapeutics in adult healthy volunteers. Rockville: U.S. Department of Health and Human Services. Retrieved from http://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf

Vaughn, A. R., Haas, K. N., Burney, W., Andersen, E., Clark, A. K., Crawford, R., & Sivamani, R. K. (2017). Potential role of curcumin against biofilm-producing organisms on the skin: A review. *Phytotherapy Research*, *31*(12), 1807–1816. https://doi.org/10.1002/ptr.5912

Wenderfer, S. E. (2012). Can inhibition of proteasomes or NF-kappaB help control idiopathic nephrotic syndrome? *Nephrology, Dialysis, Transplantation*, *27*(5), 1698–1701. https://doi.org/10.1093/ndt/gfs079

WHO (1999). *WHO monographs on selected medicinal plants*. World Health, 1(March), 390. Retrieved from http://kamillaviragzat.hu/wtDocument/browse/root/szakmai-anyagok/WHO_Monographs_vol1.pdf

Wongcharoen, W., Jai-aue, S., Phrommintikul, A., Nawarawong, W., Woragidpoonpol, S., Tepsuwan, T., … Chattipakorn, N. (2012). Effects of curcuminoids on frequency of acute myocardial infarction after coronary artery bypass grafting. *The American Journal of Cardiology*, *110*(1), 40–44. https://doi.org/10.1016/j.amjcard.2012.02.043

Yang, C., Wu, K., Li, S.-H., & You, Q. (2013). Protective effect of curcumin against cardiac dysfunction in sepsis rats. *Pharmaceutical Biology*, *51*(4), 482–487. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23336318

Zhong, F., Chen, H., Han, L., Jin, Y., & Wang, W. (2011). Curcumin attenuates lipopolysaccharide-induced renal inflammation. Biological & Pharmaceutical Bulletin. 34(2), 226–232. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21415532

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Russo ER, Facincani I, Nakazato KC, et al. Oral administration of powdered dried rhizomes of *Curcuma longa* L. (turmeric, Zingiberaceae) is effective in the treatment of doxorubicin-induced kidney injury in rats. *Phytotherapy Research*. 2018;32:2408–2416. https://doi.org/10.1002/ptr.6176