

Original Research Article

Ginger Essential Oil Ameliorates Cisplatin-Induced Nephrotoxicity in Mice

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Abstract

Purpose: To investigate the effect of ginger essential oil (GEO) in an experimental model of cisplatin-induced nephrotoxicity.

Methods: Male mice were divided into treatment six groups ($n = 7$), namely: Groups I (saline), II and III (cisplatin, 10 mg/kg, i.p.) euthanized in 3th and 6th days, respectively, and IV, V and VI (GEO, 100, 200 and 400 mg/kg/day, respectively, by gavage 3, 4, 5 and 6 days after cisplatin injection). Creatinine levels and protein/creatinine ratio were determined in plasma and urine, respectively. Bone morphogenic protein (BMP-7) and tumor necrosis factor (TNF- α) levels of kidney tissues were determined while mRNA expression levels were obtained using real-time polymerase chain reaction.

Results: GEO treatment reduced significantly creatinine levels to 0.53 ± 0.02 ; 0.48 ± 0.008 and 0.46 ± 0.02 at 100, 200 and 400 mg/kg, respectively, compared with control (0.70 ± 0.01) [$p < 0.05$] but increased protein : creatinine ratio to 0.21 ± 0.01 , 0.22 ± 0.01 , 0.24 ± 0.02 compared with control (0.06 ± 0.008) [$p < 0.05$]. Pro-inflammatory TNF- α mRNA expression was decreased to 1.46 ± 0.21 , 1.39 ± 0.19 and 1.36 ± 0.09 , at GEO doses of 100, 200 and 400 mg/kg, respectively, while anti-fibrotic BMP-7 mRNA expression increased to 2.05 ± 0.26 and 2.44 ± 0.42 at doses of 200 and 400 mg/kg, respectively, compared with control (0.59 ± 0.39 , $p < 0.05$).

Conclusion: GEO treatment attenuates cisplatin-induced nephrotoxicity, in part, by modulating some inflammatory cytokines.

Keywords: *Zingiber officinale*, Ginger, Roscoe, Essential oil, Nephrotoxicity, Cisplatin.

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INTRODUCTION

Acute kidney injury is a common disorder that develops in approximately 25 % of patients after high-dose cisplatin cancer chemotherapy [1]. Among multiple mechanisms of action, the induction of inflammatory cytokines, oxidative stress, increased creatinine levels, and apoptosis

of renal cells are involved in cisplatin-induced nephrotoxicity [2]. Cisplatin initiates the inflammatory process via tumor necrosis factor- α (TNF- α) production by resident and infiltrating immune cells [3]. Several natural products have been used to protect against toxicity induced by drugs and carcinogenic xenobiotics [4]. Different pharmacological activities have also been

reported for *Zingiber officinale* Roscoe, *Salvia officinalis* L. and *Syzygium aromaticum* L. essential oils, including antioxidant, antiinflammatory, antiviral, antibacterial and anticancer effects [5].

Furthermore, ginger extract suppresses the induction of pro-inflammatory cytokines and chemokine expression produced by synoviocytes, chondrocytes, and leukocytes in humans [6]. A previous study suggested that ginger effectively restores cisplatin-induced delays in gastric emptying in rats [7]. We recently demonstrated that ginger essential oil (GEO) presents anti-inflammatory and immunomodulatory activities in animal models [8]. Indeed, other studies have demonstrated the reno-protective effects of ginger extract in different experimental models of renal failure [9,10].

However, no studies about the reno-protective effects of GEO have been investigated. Therefore, the aim of the present study was to evaluate the effect of GEO in an experimental model of cisplatin-induced nephrotoxicity.

EXPERIMENTAL

Plant material and essential oil extraction

Fresh rhizomes of *Zingiber officinale* Roscoe were collected in June 2011 from the Prof^a Irenice Silva Medicinal Plant Garden on the campus of the State University of Maringá, Paraná, Brazil, identified, and authenticated by taxonomist Maria Aparecida Sert. Voucher specimen was deposited in the Herbarium of the Department of Botany, State University of Maringá (n^o. 11612). GEO was extracted by conventional steam distillation using a Clevenger- type apparatus for 3 h. The essential oil was stored at 4°C in dark vials, pending further tests.

Gas chromatography-mass spectrometry

Gas chromatography (GC) was performed using a Thermo Electron Corporation Focus GC model under the following conditions: DB-5 capillary column (30 m × 0.32 mm, 0.50 mm), column temperature (60 °C for 1 min to 180 °C at 3 °C/min), injector temperature (220 °C), detector temperature (220 °C), split ratio (1:10), carrier gas (He), flow rate (1.0 µl/min). The volume injected was 1 µl diluted in acetone (1:10). GC/MS analyses were performed using a Quadrupole mass spectrometer (Thermo Electron, DSQ II model) that operated at 70 V. The identification of the individual components

was based on a comparison of their GC retention indices obtained with reference to n-alkane series C₈H₁₈ – C₂₀H₄₂ (Sigma, St. Louis, MO, USA) on a DB-5 column and comparison with the mass spectra of an authentic standard purchased from Sigma-Aldrich [11]. The retention indices (RI) were obtained with reference to n-alkane C₇H₁₆-C₄₄ H₉₀ series (Supelco-Bellefonte USA, UK).

Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) was used to prove the chemical structure of the essential oil constituents identified by CG-MS. ¹H (300.06 MHz) and ¹³C NMR (75.45 MHz) spectra were recorded in a deuterated chloroform (CDCl₃) solution using a Mercury-300BB spectrometer, with δ (ppm) and spectra referenced to CDCl₃ (δ 7.27 for ¹H and δ 77.00 for ¹³C) as the internal standard.

Experimental animals

Male Swiss albino mice, 5 to 6 weeks of age and 20 - 25 g, were provided by the Central Animal House of the State University of Maringá. The animals were housed at 22 ± 2 °C under a 12h/12h light/dark cycle. All of the animals were housed in individual standard cages with free access to water and feed. The experimental protocols were approved by the Ethical Committee in Animal Experimentation of the State University of Maringá (ref no. 024/2010-CEEAA/UEM).

Drug

Cisplatin was obtained from Darrow Laboratories (Rio de Janeiro, Brazil).

Experimental design

The animals were divided into six groups. Normal group I was injected (i.p.) with saline. Cisplatin (CP) control groups (CTL) II and III were injected with CP (10 mg/kg, i.p.) and in the third and sixth days, respectively, the groups were anesthetized under ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) to evaluation of some parameters below described. Groups IV, V, VI were treated with 100, 200 and 400 mg/kg of GEO, respectively, by gavage (3, 4, 5, and 6 days after CP injection) and thereafter anesthetized as CTL groups. Each of the groups had seven animals, with the exception of Group III, which had 18 animals because some of them died between third and sixth days after cisplatin injection.

Analysis of kidney function

Blood was collected directly from the abdominal aorta and then centrifuged. The serum was separated for the determination of serum creatinine levels. Urine was collected from the bladder to measure urinary protein and creatinine levels using a commercial kit (Gold Analisa®) for calculating the urinary protein : creatinine ratio.

Quantification of mRNA

Kidney samples were quickly stored in liquid nitrogen. Total RNA was isolated from the kidney using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA), and RNA levels were determined by ultraviolet light absorbance at 260 nm using a Nanodrop 2000C spectrophotometer (Thermo Scientific). First-strand cDNAs were synthesized using MML-V reverse transcriptase (Promega, Madison, WI, USA). Reverse transcription polymerase chain reaction (RT-PCR) was performed using the SYBR Green real-time PCR assay (Applied Biosystems, USA). The following primer sequences were used: TNF- α (sense: 5'-ATG AGC ACA GAA AGC ATG ATCAGC-3'; antisense: 5'-ACG AGG AAT GAG AAG AGG GTC AGA-5'), BMP-7 (sense: 5'-AGA ATT CTT CCA CCC TGC ATA CC-3'; antisense: 5'-TCC

TTA TAG ATC CTG AAT TGG GCT-3'). mRNA expression was normalized to hypoxanthine guanine phosphoribosyl transferase (HPRT; sense: 5'-CTC ATG CAC TGA TTA TGG AGA GGA C-3'; antisense: 5'-GCA GGT CAG CAA AGA ACT TAT AGC C-3'), which was used as the housekeeping gene. All data were expressed relative to the expression of the matched controls.

Statistical analysis

The data are expressed as mean \pm SEM for each group and were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test Graph Pad Prism software (version 5). Differences were considered significant at $p < 0.05$.

RESULTS

The results of the GEO chemical analysis, retention indices, and area percentages are shown in Fig. 1 and Table 1. The chromatographic analysis identified 34 components, representing 99.3% of the total GEO.

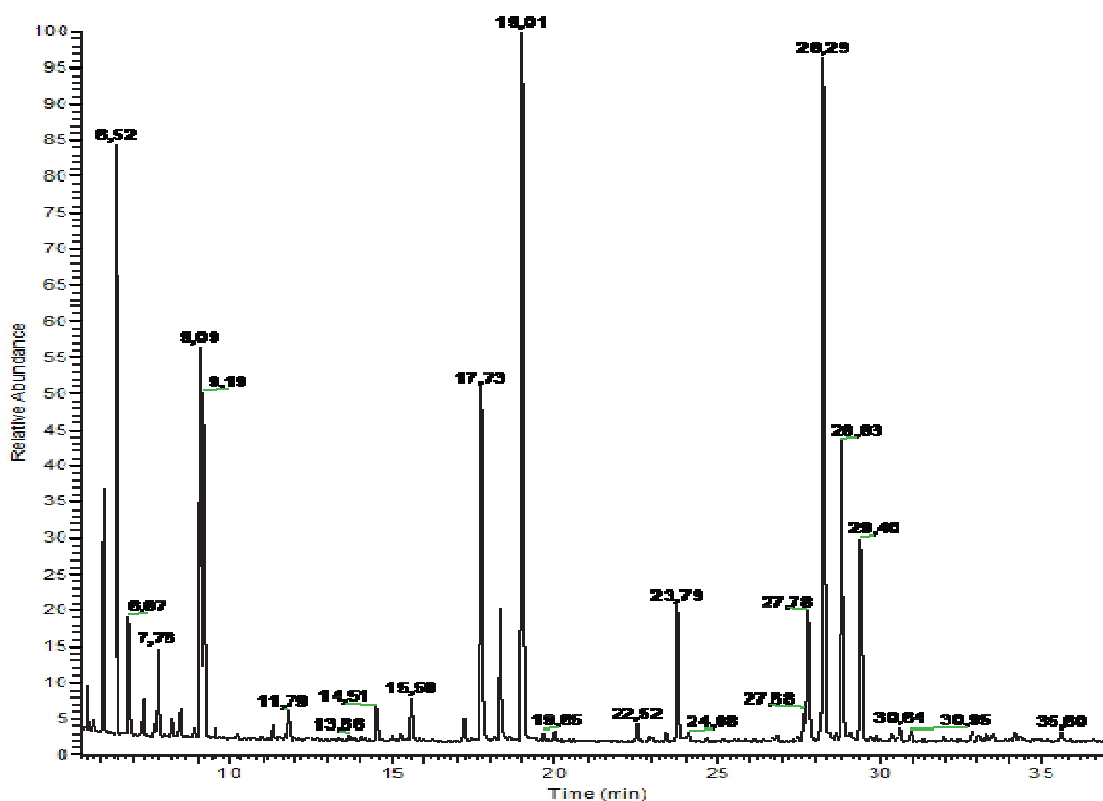


Figure 1: GC chromatogram of rhizomes of *Zingiberofficinale* Roscoe essential oil

As shown in Table 1, GEO contained a high amount of α -zingiberene (16.2%), followed by geranial (15.3 %), camphene (9.37 %), neral (7.7 %), β -phellandrene (7.6 %), and (E,E)- α -farnesene (7.4 %), representing a total of 63.5% and indicating a preponderance of terpene (monoterpene) compounds. The presence of numerous other constituents at concentrations ranging from 0.2 to 6.5 % of the essential oil was also observed.

Seric creatinine

Seric creatinine level was significantly elevated on the 3rd (0.65 ± 0.03 mg/dl) and 6th day (0.70 ± 0.01 mg/dl) after cisplatin injection compared

with 0.41 ± 0.008 mg/dl for normal control. GEO administration after cisplatin injection decreased significantly creatinine level to 0.53 ± 0.02 , 0.48 ± 0.02 and 0.46 ± 0.02 mg/dl at 100, 200 and 400 mg/kg, respectively, on the 6th day, compared with cisplatin control [$p < 0.05$], as shown in Fig 2a.

Urinary protein/creatinine ratio

Urinary protein: creatinine ratio on the 3rd (0.11 ± 0.01 mg/dl) and 6th day (0.06 ± 0.01 mg/dl) after cisplatin injection, was lower than that of normal group (0.41 ± 0.02 mg/dl), reflecting the

Table 1: Chemical composition of *Zingiber officinale* Roscoe essential oil

RI ^a	Compound	Content (%)	Technique used
953	α -pinene	3,6	GC/MS, NMR
967	Camphene	9,3	GC/MS, NMR
991	Sabinene	0,2	GC/MS, NMR
994	α -pinene	0,8	GC/MS, NMR
1005	Mycene	1,7	GC/MS, NMR
1017	α -phellandrene	0,4	GC/MS, NMR
1032	<i>p</i> -cymene	0,2	GC/MS, NMR
1036	β -phellandrene	7,6	GC/MS, NMR
1039	1,8-cineole	6,5	GC/MS, NMR
1062	α -terpinene	0,1	GC/MS, NMR
1089	Terpinolene	0,4	GC/MS, NMR
1100	Linalool	0,8	GC/MS, NMR
1165	Borneol	0,9	GC/MS, NMR
1190	α -terpineol	1,0	GC/MS, NMR
1228	Citronellol	0,5	GC/MS, NMR
1240	Neral	7,7	GC/MS, NMR
1254	Geraniol	3,4	GC/MS, NMR
1270	Geranial	15,3	GC/MS, NMR
1285	bornyl acetate	0,3	GC/MS
1353	citronellyl acetate	0,6	GC/MS
1363	no identified	0,3	-
1374	α -copaene	0,3	GC/MS, NMR
1383	geranyl acetate	3,0	GC/MS, NMR
1390	β -elemene	0,3	GC/MS
1479	germacrene D	0,7	GC/MS, NMR
1481	ar-curcumene	3,2	GC/MS, NMR
1491	β -selinene	0,8	GC/MS
1494	α -zingiberene	16,2	GC/MS, NMR
1508	(E,E)- α -farnesene	7,4	GC/MS, NMR
1522	sesquiphellandrene	4,4	GC/MS, NMR
1555	Z-Nerolidol	0,5	GC/MS
1563	E-Nerolidol	0,3	GC/MS
1612	β -bisabolol	0,3	GC/MS
1687	(E,Z)- α -farnesol	0,3	GC/MS
Total identified		99.3%	

^aRI: Retention index relative to a homologous series of *n*-alkanes on the DB-5 capillary column. MS, mass spectrometry; NMR, nuclear magnetic resonance.

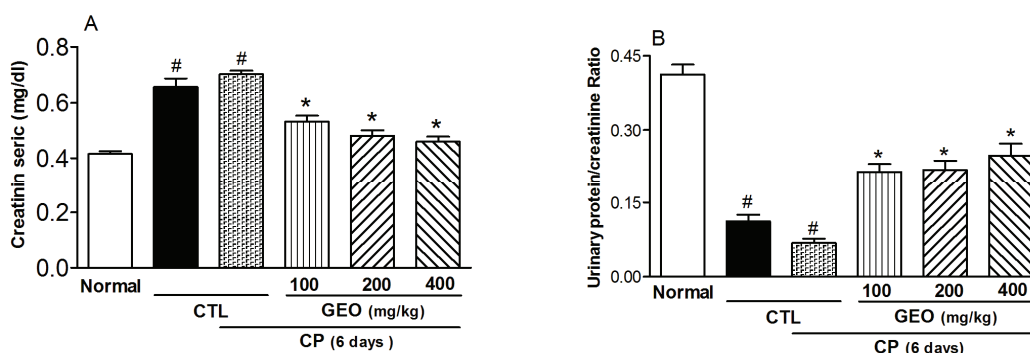


Fig 2: Effect of ginger essential oil (GEO) on cisplatin-induced renal dysfunction

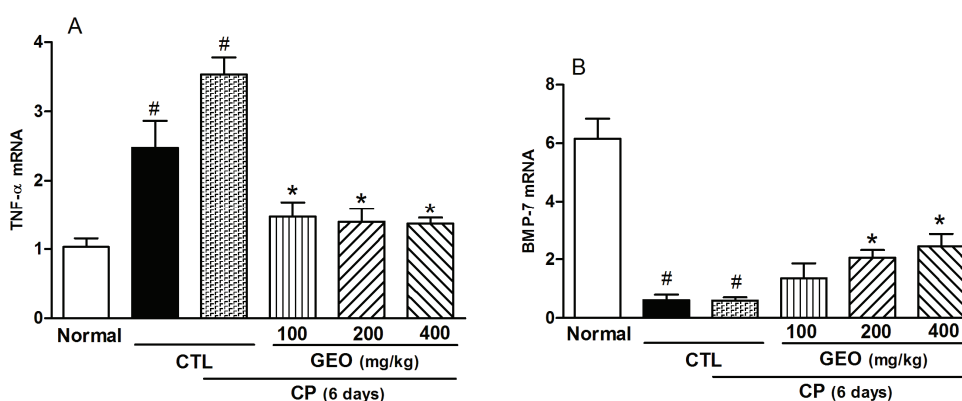


Fig 3: Changes in the expression of TNF- α and BMP-7 after administration of ginger essential oil (GEO) in a cisplatin-induced nephrotoxicity model in mice; [#] $p < 0.05$, compared with normal group; ^{*} $p < 0.05$, compared with renal injury induced by CP (6 days).

impairment of renal function. The groups that received GEO at doses of 100, 200 and 400 mg/kg after cisplatin injection increased the ratio to 0.21 ± 0.01 , 0.22 ± 0.01 , and 0.24 ± 0.02 mg/dl, respectively (Fig 2b, $p < 0.05$).

TNF- α mRNA expression levels

The TNF- α gene mRNA expression levels were markedly upregulated on 3rd day (2.47 ± 0.39) and 6th (3.53 ± 0.24) day after cisplatin injection compared with normal group (1.02 ± 0.12) [$p < 0.05$]. The groups that received GEO after cisplatin injection inhibited significantly TNF- α mRNA expression level to 1.46 ± 0.21 , 1.39 ± 0.19 and 1.36 ± 0.09 at doses of 100, 200 and 400 mg/kg, respectively, on the 6th day, compared with control (Fig 3a).

BMP-7 mRNA expression levels

BMP-7 mRNA expression levels on the 3rd (0.61 ± 0.18) and 6th days (0.59 ± 0.11) after cisplatin injection were significantly lower than that of normal control (6.16 ± 0.68). The groups that

received GEO at doses of 200 and 400 mg/kg following cisplatin injection significantly increased BMP-7 mRNA expression level to 2.05 ± 0.26 and 2.44 ± 0.42 , respectively, compared with control ($p < 0.05$). However, there was no significant increase in BMP-7 mRNA expression level in the group that received GEO at a dose of 100 mg/kg (1.35 ± 0.50), as Fig 3b shows.

DISCUSSION

The present study was aimed at investigating the nephro-protective potentials of ginger essential oil (GEO) against cisplatin-induced toxicity. Renal damage caused by cisplatin depends on the dose and duration of treatment. This study was carried out at a high dose of cisplatin (10 mg/kg/ body weight, i.p) to produce a nephrotoxic effect that corresponds to the dose of cisplatin currently being used in clinical practice. This experimental model is important because accidental cisplatin overdose can be toxic in humans and lead to renal failure [12].

Besides, GEO presents many biological activities, such as antiinflammatory, antiemetic, and antineoplastic effects, some studies have demonstrated the renoprotective effect [7,13]. However, this effect has been showed with ginger extracts in nephropathy induced by carbon tetrachloride (CCl₄) in rats [7] and against cisplatin-induced nephrotoxicity in mice [13]. In this study, seric creatinine levels were significantly elevated in the control group 3 days and 6 days after cisplatin injection compared with normal control. While GEO administration after cisplatin injection decreased creatinine levels at all test doses 6 days after cisplatin injection.

Hemmelgarn *et al* [14] showed that chronic kidney disease has a high prevalence of albuminuria and glomerular injury. In this case, albuminuria detection allows therapeutic intervention and the prevention of renal failure. Moreover, urinary protein : creatinine ratio has been shown to be an accurate and reliable method for estimating protein in the urine in many cases, such as in diabetic nephropathy and kidney-transplanted patients [15, 16]. Indeed, acute kidney injury induced by cisplatin administration in rats causes a significative reduction in the glomerular filtration rate and an increase in serum levels of urea and creatinine [17,18]. In the present study, administration of cisplatin lowered urinary protein:creatinine ratio, reflecting impairment in renal function but the ratio rose following GEO administration. These results indicate the amelioration of kidney function indicators, such as urinary protein and creatinine levels, after administration of GEO in cisplatin-treated mice, as also observed for other natural products [19,20].

Increasing evidence suggest the involvement of inflammatory processes in renal injury, including expression of genes that encode pro-inflammatory cytokines, such as TNF- α , interleukin 6 (IL-6), IL-1 β , and transforming growth factor β , which potentiate inflammation [21]. Previous studies suggested that TNF- α inhibition ameliorates cisplatin-induced renal disfunction [22]. Some natural products have been reported to suppress inflammatory processes by inhibiting the production of TNF- α and IL-1 β . In this regard, ginger extract has been demonstrated to inhibit proinflammatory cytokine (TNF- α , IL-1 β) and cyclooxygenase-2 enzyme expression in TNF- α -, IL-1 β , or lipopolysaccharide-induced THP-1 cells [23]. Our data show that TNF- α gene was markedly upregulated after cisplatin injection and that GEO administration inhibited TNF- α mRNA expression level at all the tested doses, suggesting that the reno-protective effect of GEO may be attributable

to inhibition of pro-inflammatory cytokines, such as TNF- α and IL-1 β .

Bone morphogenetic protein-7 (BMP-7) has been shown to participate in organ development, regeneration, and wound healing and has diverse biological functions. Over-expression of renal BMP-7 is capable of inhibiting TGF- β /Smad3 signaling and protecting the kidney from TGF- α -mediated renal injury [24]. BMP-7 can suppress TNF- α -induced inflammatory responses during nephropathy by inhibiting the activity of nuclear factor- κ B and increasing the activity of peroxisomal proliferator-activated receptor- γ in an immunoglobulin A-induced nephropathy model [20]. In our study, we observed that GEO treatment increases BMP-7 mRNA expression. BMP-7 has been reported to protect against ischemic injury by acting on proximal tubular epithelial cells to reduce TNF- α , IL-6, and IL-1 β expression [25].

CONCLUSION

GEO treatment has beneficial effects in an experimental mice model of cisplatin-induced nephrotoxicity, as reflected by decreased creatinine levels and increase in the protein : creatinine ratio. GEO also inhibits the expression of pro-inflammatory TNF- α cytokine levels and protects against ischemic injury by increasing BMP-7 expression. Thus, GEO treatment may possess potential benefits to renal patients. However, further investigations are essential to elucidate the mechanism by which GEO treatment ameliorates the nephrotoxicity.

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