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Protective Effect of *Zingiber officinale* on Gentamicin-Induced Nephrotoxicity in Rats

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Abstract: The present study was undertaken to evaluate the effect of ethyl acetate extract of fresh rhizomes of *Zingiber officinale* and dried fresh juice of fresh rhizomes of *Zingiber officinale* for its protective effects on gentamicin-induced nephrotoxicity in rats. Nephrotoxicity was induced in Wistar rats by intraperitoneal administration of gentamicin 100 mg/kg/day for eight days. Effect of concurrent administration of ethyl acetate extract and fresh juice extract of *Zingiber officinale* at a dose of 200 mg/kg/day given by oral route was determined using serum creatinine, serum uric acid, blood urea nitrogen and serum urea as indicators of kidney damage. The study groups contained six rats in each group. It was observed that the ethyl acetate extract and fresh juice extract of *Zingiber officinale* significantly protect rat kidneys from gentamicin-induced histopathological changes. Gentamicin-induced glomerular congestion, peritubular and blood vessel congestion, epithelial desquamation, accumulation of inflammatory cells and necrosis of the kidney cells were found to be reduced in the groups receiving the ethyl acetate and dried fresh juice extract of *Zingiber officinale* along with gentamicin. The extracts also normalized the gentamicin-induced increase in serum creatinine, serum uric acid, blood urea nitrogen and serum urea levels. This is also evidenced by the histopathological studies. Both the extracts possess significant nephroprotective activity.

Key words: *Zingiber officinale*, gentamicin, nephrotoxicity, ethyl acetate, dried fresh juice extract

INTRODUCTION

Toxic effects on the kidney related to medications are both common and expected, given the kidney's roles in plasma filtration and maintenance of metabolic homeostasis. The renal vascular bed is exposed to a quarter of resting cardiac output. As such, glomerular, tubular and renal interstitial cells frequently encounter significant concentrations of medications and their metabolites, which can induce changes in kidney function and structure. Renal toxicity can be a result of hemodynamic changes, direct injury to cells and tissue, inflammatory tissue injury and/or obstruction of renal excretion. Markers of early injury are being investigated (Han and Bonventre, 2004). In the meantime, however, subtle renal damage (e.g., tubulopathy, acid-base abnormalities, electrolyte imbalances and disorders of water balance) and mild urinary sediment abnormalities associated with commonly used medications are frequently unrecognized. Detection is often delayed until an overt change in renal functional capacity is measured as an increase in serum blood urea nitrogen or creatinine.

The true incidence of drug-induced nephrotoxicity is therefore difficult to determine. Studies that evaluated episodes of Acute Tubular Necrosis (ATN) or Acute Interstitial Nephritis (AIN) attributed to medication determined the incidence to be as high as 18.3%. The incidence of nephrotoxic injury due to antibiotics (e.g., aminoglycosides) has been reported to be up to 36% (Kleinknecht *et al*., 1987; Kaloyanides *et al*., 2001). Most episodes of drug-induced renal dysfunction are reversible, with function returning to baseline when the medication is discontinued. Chronic renal injury can, however, be induced by some medications, leading to chronic tubulointerstitial inflammation, papillary necrosis or prolonged proteinuria (Kleinknecht, 1995; Klinkhoff and Teufel, 1997). Heightened physician awareness is necessary if renal injury and associated morbidity from renal failure are to be prevented.

Zingiber officinalis, Roscoe is a rhizome that is widely used as culinary herb and herbal remedy for some common ailments. It is used as carminative, antipyretic, antiemetic in pregnancy and as anticancer adjunct (Evans and Trease, 1979). It also ameliorates motion sickness and it is a known thromboxane synthesis and platelet aggregation inhibitors and diaphoretic agent (Evans and Trease, 1979). It contains about 1-2% of volatile oil and 5-8% of resinous matter, starch and

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mucilage (Evans and Trease, 1979). The volatile oil contains monoterpenes, sesquiterpenes and sesquiterpene alcohol zingiberol (Evans and Trease, 1979), gingerol and shagoals. Most of the pharmacologically active constituents reside in the volatile oils. Gingerols have cardio tonic (Kobayashi *et al*., 1988) analgesic, anti-inflammatory (Young *et al*., 2005), antipyretic (Yoshikawa *et al*., 1993) and antibacterial effects both *in vitro* and *in vivo* (Mascolo *et al*., 1989). Shagoal has antiemetic, antispasmodic, anxiolytic and anticonvulsant activity (Vishwakarma *et al*., 2002). Scientific reports show that it is also used for conditions such as anti-ulcerogenic (Al-Yahya *et al*., 1989), anti-diabetic (Akhani *et al*., 2004), anti-oxidant (Ahmad *et al*., 2006) and anti-hepatotoxic (Yemitan and Izegbu, 2006) activities. However, systematic and scientific reports on the investigation of *Z. officinale* for its effects on renal function are scarce. In the present study, an effort has been made to evaluate the effects of the ethyl acetate extract and dried fresh juice of fresh rhizomes of *Z. officinale* on gentamicin-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Preparation of the plant material: *Zingiber officinale* rhizomes were collected from the local market of Rangareddy District, Hyderabad, in the month of February-March, the botanical authentication was done by the Department of Botany, Osmania University, Hyderabad and voucher specimen (MRCP-104) is lodged in our research laboratory for the future reference. The fresh rhizomes were sliced using a home slicer and the slices obtained were shade-dried, pulverized and passed through a 20-mesh sieve. The dried, coarsely powdered plant material was extracted with ethyl acetate using a Soxhlet apparatus. The solvent was evaporated under vacuum, which gave semisolid mass (ZOEAE, yield: 26% w/w) with respect to the dried powder. Also the rhizomes were cut into small pieces and fed to a juicer to collect the juice and the collected juice was filtered and vacuum dried to obtain the *Z. officinale* fresh juice extract (ZOFJE, yield: 19% w/w). Oral suspensions containing 200 mg mL^{-1} of the ZOEAE and ZOFJE were prepared in 1% w/v gum acacia.

Experimental animals: Healthy, male Wistar rats each weighing 150-200 g were used for this study. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3°C and 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum*. The Institutional Animal Ethical Committee (IAEC) of Malla Reddy College of Pharmacy, Hyderabad, with Reg. No. 1217/a/08/CPCSEA, approved the study.

Evaluation of nephroprotective activity: Twenty-four male Wistar rats were assigned to four groups: group 1: control group, group 2: Gentamicin-treated group, group 3: Gentamicin as well as ethyl acetate extract of rhizomes of *Z. officinale*-treated group and, group 4: Gentamicin as well as dried fresh juice extract of rhizomes of *Z. officinale*-treated group, each group containing six rats. The gentamicin-treated group received 100 mg/kg/day gentamicin (Hi Media Laboratories, India) by the intraperitoneal (i.p.) route (Azhar-Alam *et al*., 2005). Group 3 received 100 mg/kg/day gentamicin i.p. and 200 mg/kg/day of the ZOEAE p.o. for eight days and group 4 received 100 mg/kg/day gentamicin i.p. and 200 mg/kg/day of the ZOFJE p.o. for eight days. Rats in the control group were given sterile saline solution i.p. for the same number of days. After dosing on the 8th day, blood samples were collected via cardiac puncture method at the end of these 24 h. The serum was rapidly separated and processed for determination of serum creatinine, serum uric acid, Blood Urea Nitrogen (BUN) and serum urea using commercially available kits of Span Diagnostics Ltd., India (Shirwaikar *et al*., 2004). Changes in kidney weight were recorded. Three rats per group were sacrificed and both kidneys were isolated from each rat (Annie *et al*., 2005). The kidneys were weighed and processed for histopathological examination (Erdem *et al*., 2000).

Histopathological examination: The kidneys were sectioned longitudinally in two halves and were kept in 10% neutral formalin solution (Ogeturk *et al*., 2005). Both kidneys were processed and embedded in paraffin wax and sections were taken using a microtome. The sections were stained with hematoxylin and eosin and were observed under a computerized light microscope.

Statistical analysis: The data obtained was analyzed using one-way ANOVA followed by Dunnette's multiple comparison test. The $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Serum creatinine, serum uric acid, blood urea nitrogen, serum urea and the weights of the kidneys were found to be significantly increased in rats treated with only gentamicin; whereas treatment with the ZOEAE and ZOFJE was found to protect the rats from such effects of gentamicin (Table 1).

Control rats showed normal glomeruli with an intact Bowman's capsule and proximal convoluted capsule (Fig. 1). Rats treated with gentamicin, showed tubular epithelial loss with intense granular degeneration involving >50% renal cortex. In addition to the tubular epithelial loss, some of the tubular epithelium contains

Table 1: Parameters studied for the nephroprotective activity of the ethyl acetate and dried fresh juice extracts of rhizomes of *Zingiber officinale*

Group	Serum creatinine	Serum uric acid	Blood urea nitrogen	Serum urea	Weight of kidney (g)
	---------- (mg mL^{-1}) ----------				
Control	1.49±0.021	3.64±0.91	18.65±0.95	32.00±3.54	1.23±0.34
Gentamicin	3.37±0.45*	5.15±1.09*	45.97±1.24*	97.31±2.98*	2.21±0.92*
Treatment 1	1.56±0.51**	2.51±1.54*	24.06±1.54**	50.40±3.12*	1.51±0.23**
Treatment 2	1.86±0.71**	3.78±2.05	28.64±2.12*	61.67±2.76*	1.73±0.98*

Treatment 1: Ethyl acetate extract treated group, Treatment 2: Fresh juice extract treated group. Values are expressed as Mean±SEM. n = 6 rats in each group. *p<0.05 compared to control group, **p<0.001 compared to gentamicin treated control group

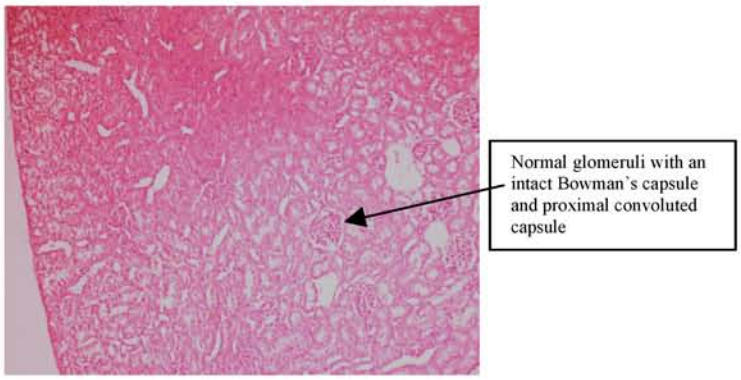

Fig. 1: Kidney tissue of negative control treatment. Stain haematoxylin and eosin at magnification 40X

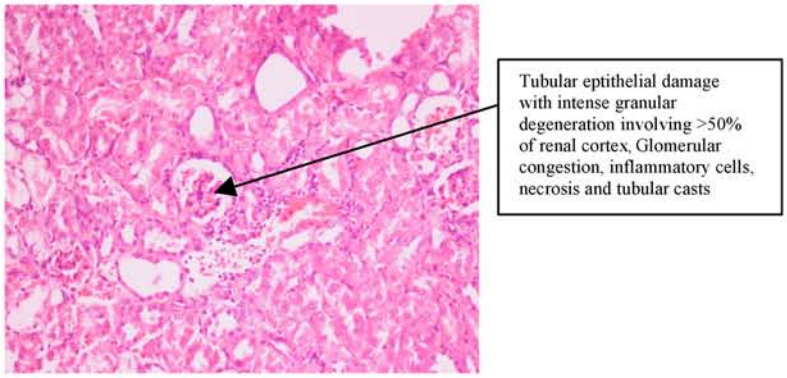

Fig. 2: Kidney tissue of animal treated with Gentamicin (positive control). Stained with haematoxylin and eosin, at magnification 200X

tubular casts and blood vessel congestion and result in the presence of inflammatory cells in kidney sections (Fig. 2). The histomorphology of rats treated with ethyl acetate extract plus gentamicin showed moderate tubular epithelial degeneration with desquamation in patchy areas of the renal cortex (Fig. 3). Concurrent treatment of rats with the dried fresh juice extract (Fig. 4) also was found to reduce such changes moderately in kidney histology induced by gentamicin (Table 2).

Nephrotoxicity induced by gentamicin has been found to be a complex phenomenon characterized by an increase in serum creatinine, urea, uric acid and BUN levels and severe proximal renal tubular necrosis followed by renal failure (Al-Majed *et al.*, 2002). The reduction in glomerular filtration rate, which is indicated by the increase in serum creatinine level, would be accompanied by an increase in Serum urea, uric acid and BUN levels when a marked renal parenchymal injury occurs (Erdem *et al.*, 2000). *Zingiber officinale* treatment did not produce detectable changes in serum Creatinine, urea, uric acid and BUN levels in normal rats, but it suppressed the gentamicin-induced increases in serum creatinine, urea, uric acid and BUN levels with both the extracts. There was also significant increase in the weight of the kidney in the gentamicin treated group. This was brought back to normal levels with the treatment of the extracts.

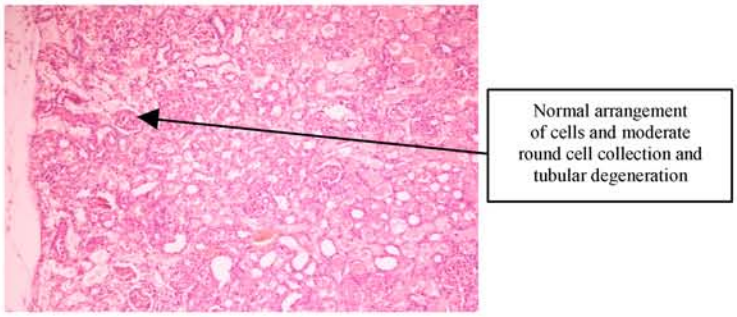

Fig. 3: Kidney tissue of ethyl acetate extract-treated animals. Stained with haematoxylin and eosin, at magnification 200X

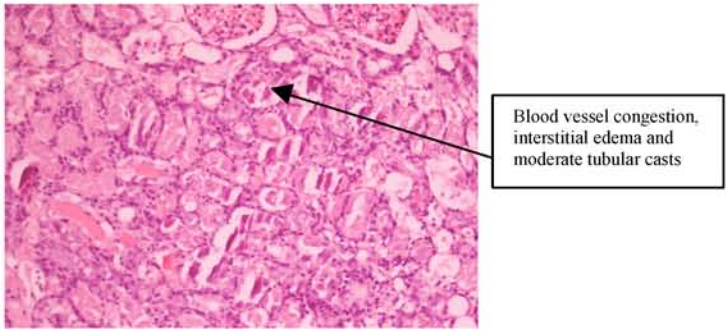

Fig. 4: Kidney tissue of fresh juice extract-treated animals. Stained with haematoxylin and eosin, at magnification 100X

Table 2: Histopathological features of the kidneys of rats of different treatment groups

Histopathological feature	Control	Gentamicin treated	Gentamicin and ethyl acetate extract treated	Gentamicin and fresh juice extract treated
Glomerular congestion	-	+++	+	++
Blood vessel congestion	-	++	--	+
Interstitial edema	-	++	--	+
Inflammatory cells	-	++	--	+
Necrosis	-	++	--	--
Tubular casts	-	+++	+	+

++: Presence, --: Absence

A huge body of experimental evidence has indicated that Reactive Oxygen Species (ROS) are involved in the pathogenesis of gentamicin nephrotoxicity (Reiter *et al.*, 2002; Al-Majed *et al.*, 2002). The impairment of renal mitochondrial antioxidant system by gentamicin intoxication (Ahmad *et al.*, 2006) supports the role of ROS in gentamicin-induced renal damage. Given the crucial role of maintenance of mitochondrial antioxidant status in cell survival (Pascoe and Reed, 1989), the ability of *Z. officinale* treatment to enhance renal mitochondrial antioxidant system (Stoilova *et al.*, 2007) is likely related to the nephroprotection against gentamicin toxicity.

In conclusion, *Z. officinale* extracts contain phytoconstituents-flavonoids which could enhance renal mitochondrial antioxidant system (Hollman and Katan, 1999), thereby protecting against gentamicin nephrotoxicity.

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