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Herbal formulation (Kidney Revival) ameliorated gentamicin induced kidney injury in rats

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Original Article

Abstract:

Background and aims: Kidney injury is associated with serious short and long-term morbidities, increased risk of death and stupendous healthcare costs. The objective of this study was to investigate the therapeutic role of Kidney Revival (KR), an herbal formulation, on gentamicin-induced kidney injury in animals.

Methods: The antioxidant properties of KR were studied using the DPPH assay and hydrogen peroxide, nitric oxide and superoxide radical scavenging. Moreover, the therapeutic application of KR was studied in acute oral toxicity in mice and gentamicin-induced renal toxicity in rats. All clinical, pathological and biochemical features related to kidney injury were carefully studied.

Results: *In vitro* experiments showed free radical scavenging properties of KR. Acute oral toxicity investigations revealed that KR was safe up to 2 ml/kg. Treatment with KR in gentamicin-induced nephrotoxic animals showed a significant ($p<0.05$) and dose-dependent enhancement of excretion of urea and creatinine. It also maintained electrolyte (sodium, potassium and chloride) balance in blood and counteracted

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oxidative stress in renal tissues. Histopathological findings also corroborated the results.

Conclusion: Enhancement of creatinine and urea excretion indicated that KR acted directly on renal tubules to modify glomerular filtration rate and its excretory functions.

Keywords: Kidney, glomerular filtration rate, Gentamicin, antioxidant, creatinine, herbs

INTRODUCTION

Acute kidney injury (AKI) is a syndrome characterized by a rapid deterioration of renal function (lasting for few hours to days), manifested by elevation in serum creatinine and blood urea nitrogen (BUN) and associated with serious short and long-term morbidities (1-2). AKI affects nearly 10% of hospital admissions with overall mortality of 20-50% in ICU patients. The incidence of AKI has doubled over the last 15 years. Besides this, there are approximately 7.85 million people suffering from chronic kidney disease (CKD) in India (3-4). However, there are very few treatments, and arguably no safe modern medicine, for kidney diseases. Unfortunately, modern medicines cause AKI in approximately 20% of the community and hospitals (5).

The kidney can be damaged by poor blood flow, mitochondrial dysfunction, overload due to the high total level of toxins, which may not be independently highly toxic but become problematic when the their total level is high, either indirectly by toxins that cause general tissue damage or directly by toxins that are specifically harmful to the kidney tissues (6). Immunosuppressants, aminoglycosides, aminonucleosides, antibiotics and angiotensin-converting enzyme (ACE) inhibitors potentially lead to nephrotoxicity (5). Furthermore, exposure to chemicals such as ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals including lead, mercury, arsenic and cadmium also induce nephrotoxicity (7). Most of the drugs found to cause nephrotoxicity lead to the condition through one or more common pathogenic mechanisms including altered intraglomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis and thrombotic microangiopathy (8).

Any successful prevention requires knowledge about patient-

related risk factors, drug-related risk factors, and early intervention. Hence, searching for novel and comparably safer drugs for the prevention and the treatment of kidney diseases is utmost urgent. In traditional system of medicine, many herbs and their active ingredients have been used for the prevention and treatment of kidney diseases (9-10). Kidney Revival (KR) is an herbal formulation prepared with active ingredients of purified water extracts of *Boerhaavia verticillata, Crataeva nurvala, Tinospora cordifolia, Emblica officinalis, Tribulus terrestris, Solanum nigrum, Nelumbo nucifera* and honey. Though KR is claimed to restore renal function, there has yet been no scientific report. Therefore, it was decided to study the pharmacodynamic action of KR on kidney diseases, particularly drug-induced kidney damage, in animals.

MATERIALS AND METHODS

Test material:

Each 10 ml KR contains purified water extracts of *B. verticillata* Rt. (85 mg), *C. nurvala* St. Bk. (85 mg), *T. cordifolia* St. (50 mg), *E. officinalis* Fr. P (30 mg), *T. terrestris* Fr. (50 mg), *S. nigrum* W.P. (30 mg), *N. nucifera* Fl. (20 mg) and honey *q.s.*

Animals:

Adult female Swiss mice weighing 20-25 g and adult male Wistar rats weighing 180-190 g were housed in polypropylene cages for rodents and fed with balanced pellet diet and water *ad libitum*. The room's air was changed 10-15 times per hour, its temperature set at 20-25ºC, its relative humidity at 30-65% and its illumination at 12:12 light–dark cycle using artificial fluorescent (11). All experiments were conducted after institutional animal research ethics committee approved the study protocol.

In vitro antioxidant activities:

DPPH radical scavenging:

KR was dissolved in 80% hydro-methanol solution at different (10-100 µl/ml) concentrations. Then, 0.1 ml of KR was dissolved in 3.9 ml of 0.135 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution and left in the dark for 30 min. The optical density was determined at 517 nm (12). The radical scavenging activity of KR was expressed as the half maximal inhibitory concentration (IC_{50}).

Hydrogen peroxide scavenging:

KR was dissolved in phosphate buffer (50 mM, pH 7.4) at different concentrations. Two ml of different concentrations of KR was dissolved in 0.6 ml of 40 mM H_2O_2 and 10 min later, its optical density was read at 532 nm (12). The inhibition percentage of KR was calculated.

Nitric oxide radical scavenging:

KR was dissolved in phosphate buffer saline (1M, pH 7.2) at different concentrations. 0.5 ml of different concentration of KR was dissolved in 2 ml of 10mM sodium nitroprusside and incubated at 25ºC for 150 min. Afterwards, 0.5 ml of the reaction solution containing nitrite was dissolved in 1 ml of 0.33% sulfanilic acid solution and left for 5 min. Then, 1 ml of 1% naphthylethylene diamine dihydrochloride was added, mixed and left for further 30 min (12). The optical density was read at 540 nm and the IC_{50} was calculated.

Superoxide anion radical scavenging:

KR was dissolved in 0.1 M Tris-HCl (pH 7.4) at different concentrations. To 3 ml of Tris-HCl buffer, 0.75 ml of nitro blue tetrazolium (300 µM), 0.75 ml of NADH (936 µM) solution and 0.3 ml of KR were sequentially added and mixed. Thereafter, 0.75 ml of phenazine methosulphate (120 µM) was added to the mixture and exposed to light at room temperature for 5 min (12). The optical absorbance was read at 560 nm and the inhibition percentage was calculated.

Acute oral toxicity studies in mice:

KR was examined for safety according to the guidelines of Organisation for Economic Co-operation and Development (OECD) No. 423 (13). To this end, KR was given orally to 18 h fasted female Swiss mice (weighing 20-25g) in an arithmetically progressive manner at 500 mg/kg, 1000 mg/kg, 1500 mg/kg and 2000 mg/kg, in single doses and the animals were meticulously observed for three days. The rate of mortality up to three days was recorded for determination of 50% lethal concentration of KR.

Gentamicin-induced renal injury in rats:

Male Wistar rats (180-190 g) were divided into 4 groups (n=6): (i) normal control; (ii) gentamicin-induced control; (iii) gentamicin-induced and KR (100

mg/kg)-treated; and (iv) gentamicin-induced and KR (200 mg/kg)-treated. Gentamicin at 100 mg/kg was given intraperitoneally to all rats (except normal control) daily for five consecutive days to induce nephrotoxicity (14-15). *KR was given orally* at two different single doses, 100 and 200 mg/kg, daily from day 6 until day 15 after gentamicin injection. On day 15, animals were individually placed in separate metabolic cages for four hours for collection of urine samples. Urine output and microalbumin, urea and creatinine levels in urine were measured (16). On the next day, blood was collected from the retro-orbital plexus of the animals. Protein, albumin, urea, creatinine, sodium, potassium and chloride in blood were estimated spectrophotometrically (16-17). Thereafter, all animals were sacrificed under deep anaesthesia and small sections of renal tissues were homogenized (10%) in phosphate buffer saline to measure protein (18), lipid peroxide (LPO) (19), glutathione (GSH) (20), catalase (20) and superoxide dismutase (SOD) (21) as per a procedure previously described. Moreover, sections of renal tissues were processed for histological investigations (15).

Statistical analysis:

Data were presented as mean ± SD for the indicated number of independently performed experiments. Statistical significance was investigated using t-test in SPSS (version 20, IBM, USA). P value less than 0.05 was considered as significance level.

RESULTS AND DISCUSSION

The free radicals such as superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet}$), and the non-radical oxidants hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) may be involved in renal injury. Oxidative stress rapidly progresses through inflammation, cardiovascular complications, fibrosis and apoptosis as well as through damage to the glomerular filtration barrier (22). Superoxide anion is a significant precursor of reactive oxygen species (ROS) such as H_2O_2 and $OH^{\bullet}$, and can react with other radicals including $NO^{\bullet}$ to form reactive nitrogen species (RNS)

such as $ONOO^-$. Cellular $O_2^{\bullet-}$ is produced by dysfunctional mitochondria in hypoxia, ischemia or toxicity and can induce lipid and protein oxidation in renal injury (23). Natural and dietary antioxidants play a substantial role in protecting renal injury against oxidative stress (24). In the present study, KR showed strong antioxidant properties. It not only scavenges non-radical DPPH but also strongly reacts with some radicals of ROS origin such as $O_2^{\bullet-}$ and H_2O_2 and some radicals of NOS origin such as $ONOO^{-/}NO^{\bullet}$ (Table 1). The active ingredients of *E. officinalis, T. cordifolia, T. terrestris, S. nigrum* present in KR have been reported to exhibit strong antioxidant properties (9).

Kidney damage may be acute or chronic and is traditionally diagnosed using blood markers such as BUN and creatinine levels that rise only after considerable renal tissue damage. Gentamicin (aminoglycoside) is a widely prescribed antibiotic for treating infections, but it is associated with serious adverse effects for kidney injury, limiting its long-term clinical use (25). The pathogenesis of aminoglycosides nephrotoxicity is a two-step process. The first step entails the transportation and accretion of antibiotics in high concentration by renal proximal tubular cells, while the second step involves adverse interaction between these polycationic drugs, leading to cell damage (26). Gentamicin-induced nephrotoxicity model is one of the most commonly used animal models to study renoprotective drugs and phytopharmaceuticals (15). In the present study, the renoprotective efficacy of KR was assessed in animal model of gentamicin-induced AKI.

Glomerular filtration rate (GFR) is a key criterion to determine kidney function. Assessment of metabolites in urine for diagnosis and treatment of AKI, therefore, is strongly recommended. In this study, significant elevation of urine volume (112%) and microalbumin (5.3 times) in urine was noticed. Besides this, excretion of BUN (-58%) was diminished. The renal clearance, or more specifically creatinine clearance, drastically changed. All these phenomena indicate moderate to severe kidney damage in gentamicin-induced animals (Table 2).

However higher amounts of microalbumin excretion confined injuries and structural damage in the S1 and S2 segment of proximal convoluted tubules (27). Gentamicin-receiving rats exhibited a 3.75-fold increase in creatinine and 2-fold increase in urea in blood. Moreover, hyperkalemia followed by significant loss of sodium and chloride established its electrolyte imbalances (Table 3). Loss of protein and albumin in urine also indicated partial failure in renal tubular reabsorption process (28).

Safety studies demonstrated that KR was safe for oral use up to a dose of 2 g/kg in mice. Two effective doses, 100 mg/kg and 200 mg/kg, were selected for rats on the basis of maximum tolerable oral doses obtained from acute toxicity studies. Therapeutic application of KR (100 mg/kg and 200 mg/kg, PO) in gentamicin-receiving animals significantly ameliorated kidney injuries. KR not only significantly enhanced the urea and creatinine excretion through urine, but also reduced urea and creatinine in blood. Furthermore, KR significantly maintained the balances of electrolytes. It helps in reabsorption of sodium and chloride by proximal part of renal tubules through exchange/elimination of potassium in blood (Tables 2-3). Hyperkalemia is one of the most common and life-threatening electrolyte disorders in kidney diseases. Significant enhancement of creatinine clearance indicated that KR acted directly on renal tubules to modify GFR and its excretory functions (Fig 1).

Fig. 1: Creatinine Clearance

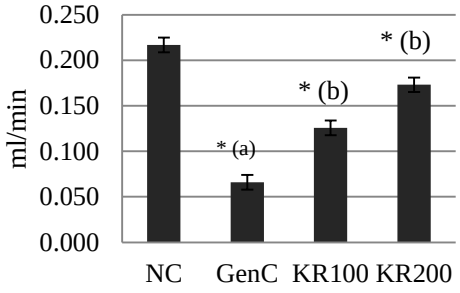

Figure 1. Kidney Revival Effect on Creatinine Clearance in Gentamicin-induced Kidney Injury in Rats

(a) Compared to normal control; (b) compared to gentamicin control; * $p<0.05$.

N-acetylcysteine (NAC) has been reported to guard the kidney from injury induced by contrast media, ischemia and toxins. In one study, NAC, as with KR, also showed protective effects on gentamicin-mediated nephropathy, with the mechanisms of the protective effects

potentially being related to interference with the peroxynitrite-related pathways (29). In the present study, KR also showed nitric oxide radical scavenging activity. In all these studies, GFR served as the surrogate marker of kidney injury and serum creatinine changes were studied as indices of GFR (14, 25, 30).

Much evidence has suggested the noticeable role of ROS in gentamicin-induced nephrotoxicity (31). Earlier studies demonstrated antioxidant properties of KR through *in vitro* experiments. Similarly, in *in vivo* experiments, KR significantly and dose-dependently ameliorated oxidative stress by altering lipid peroxides, glutathione, catalase and superoxide dismutase in renal tissues (Table 4). Therefore, the therapeutic effect of KR may be derived from its antioxidant activities including non-radical oxidants and/or protecting GSH against peroxidase activity or protecting against renal cell damage. The constituents of KR like *B. verticillata*, *C. nurvala*, *T. cordifolia*, *E. officinalis*, *T. terrestris*, *S. nigrum*, *N. nucifera* and honey have been reported to have renoprotective and therapeutic effects on nephropathies (32-38). Histopathological findings also corroborated previous observations. Tubular damage including loss of brush borders, tubular debris, congested proximal tubules, degeneration of bowman capsule and glomerulus, tubular necrosis and vast cellular vacuolations were noticed in the renal biopsy samples of gentamicin-induced control animals (Fig. 2). KR-treated groups exhibited markedly less histological damage than gentamicin-receiving rats, indicating the renoprotective action of the formulation.

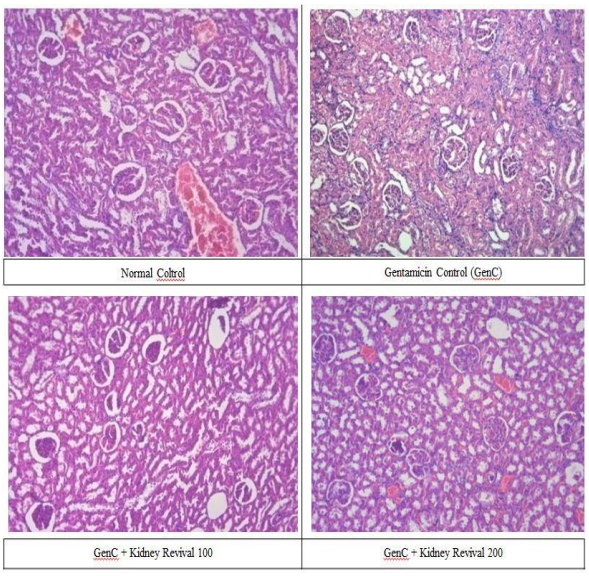

Figure 2. Histopathology of Renal Tissues
(H&E staining, magnification 10x)

Table 1. *In vitro* antioxidant activities of Kidney Revival

50% Inhibition Concentration (IC_{50}) of Kidney Revival			
DPPH Scavenging activity	Hydrogen peroxide scavenging activity	Nitric oxide scavenging activity	Superoxide scavenging activity
36.3886±1.042	24.1587±0.9853	26.0238±1.227	20.5152±1.6809

N=3; Mean ± SD; Conc. of Kidney Revival= µl/ml

Table 2. Urine analysis on gentamicin induced nephrotoxic rats

	NC	GenC	GenC + KR100	GenC + KR200
Urine Volume (ml/24 h)	6.85±0.62	14.52±1.87$^{(a)*}$	10.74±1.26$^{(b)*}$	9.16±1.55$^{(b)*}$
BUN (mg/dl)	178.6±5.48	74.86±6.85$^{(a)*}$	132.72±4.34$^{(b)*}$	152.28±4.89$^{(b)*}$
Creatinine (mg/dl)	26.44±6.15	14.28±5.44$^{(a)*}$	19.56±4.19$^{(b)*}$	22.85±4.53$^{(b)*}$
Micro Albumin (mg/dl)	0.67±0.27	4.24±0.152$^{(a)*}$	2.92±0.106$^{(b)*}$	1.73±0.093$^{(b)*}$

N=6; Mean ± SD; NC=Normal Control; GenC= Gentamicin induced Control; KR100= Kidney Revival 100 mg/kg; KR200= Kidney Revival 200 mg/kg; (a) compared to NC and (b) compared to GenC; t-test between groups; * indicate $p<0.05$

Table 3. Blood analysis on gentamicin induced nephrotoxic rats

	NC	GenC	GenC + KR100	GenC + KR200
Protein (g/dl)	8.14±0.58	5.27±0.72$^{(a)*}$	6.12±0.63$^{(b)*}$	6.93±0.81$^{(b)*}$
Albumin (g/dl)	4.12±0.27	2.72±0.43$^{(a)*}$	3.46±0.51$^{(b)*}$	3.81±0.35$^{(b)*}$
Urea (mg/dl)	34.16±3.86	71.73±8.22$^{(a)*}$	49.15±6.08$^{(b)*}$	41.69±5.59$^{(b)*}$
Creatinine (mg/dl)	0.58±0.05	2.18±0.94$^{(a)*}$	1.16±0.39$^{(b)*}$	0.84±0.52$^{(b)*}$
Sodium (mEqM/L)	138.3±3.64	118.4±6.82$^{(a)*}$	125.8±5.59$^{(b)*}$	134.2±5.22$^{(b)*}$
Potassium (mEqM/L)	4.32±0.45	5.78±0.82$^{(a)*}$	4.94±0.71$^{(b)*}$	4.75±0.63$^{(b)*}$
Chloride (mEqM/L)	115.24±3.08	68.47±7.62$^{(a)*}$	93.04±6.15$^{(b)*}$	102.97±5.24$^{(b)*}$

N=6; Mean±SD; NC=Normal Control; GenC= Gentamicin induced Control; KR100= Kidney Revival 100 mg/kg; KR200= Kidney Revival 200 mg/kg; (a) compared to NC and (b) compared to GenC; t-test between groups; * indicate $p<0.05$

Table 4. Antioxidants of renal tissue on gentamicin induced nephrotoxic rats

	NC	GenC	GenC + KR100	GenC + KR200
Lipid Peroxides (nM MDA/mg protein)	0.052±0.007	1.764±0.369$^{(a)*}$	0.829±0.062$^{(b)*}$	0.385±0.071$^{(b)*}$
Catalase (µM H_2O_2 decomposed/ min/ mg protein)	4.05±0.08	1.35±0.16$^{(a)*}$	2.25±0.36$^{(b)*}$	3.05±0.42$^{(b)*}$
Glutathione (nM/mg protein)	1.86±0.28	0.56±0.08$^{(a)*}$	0.92±0.07$^{(b)*}$	1.33±0.09$^{(b)*}$
Superoxide dismutase (U/mg protein)	15.52±0.95	6.25±0.43$^{(a)*}$	9.14±0.82$^{(b)*}$	12.64±0.77$^{(b)*}$

N=6; Mean ± SD; NC=Normal Control; GenC= Gentamicin induced Control; KR100= Kidney Revival 100 mg/kg; KR200= Kidney Revival 200 mg/kg; (a) compared to NC and (b) compared to GenC; t-test between groups; * indicate $p<0.05$

CONCLUSION

Taken together, it can be argued that KR has the potential to heal the damages of renal tissues due to gentamicin-induced oxidative stress. It not only provides the antioxidants support to renal tissues to repair their structural damage but also rearranges the functional disturbances caused by gentamicin.

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