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Research Article
Effect of Chrysin on Gentamicin-induced Nephrotoxicity in Laboratory Animals

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Abstract

Background: Clinically Gentamicin (GM) is commonly used aminoglycoside antibiotic for the treatment of life-threatening Gram-negative bacterial infections, but the nephrotoxic potential of drug limit its clinical interest. Chrysin a plant flavonoid possess potent antioxidant and anti-inflammatory activity. **Objective:** To investigate the potential of chrysin against GM-induced nephrotoxicity. **Materials and Methods:** Nephrotoxicity was induced in male Sprague-Dawley rats (220-250 g) by intraperitoneal administration of gentamicin (120 mg kg^{-1}) for 7 consecutive days. Rats were either treated with chrysin (10, 20 and 40 mg kg^{-1}, p.o.) or methylprednisolone (12.5 mg kg^{-1}, i.p.) or vehicle distilled water (10 mg kg^{-1}, p.o.) for the 7 days. Various biochemical, molecular and histological parameters were assessed in serum and kidney. **Results:** The GM-administration significantly increased ($p<0.001$) the serum creatinine and Blood Urea Nitrogen (BUN) as well as renal malonaldehyde (MDA), Nitric Oxide (NO) along with renal Kidney Injury Molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) mRNA expressions. In addition, GM also significantly decreased ($p<0.001$) the renal superoxide dismutase (SOD), reduced glutathione (GSH) and mitochondrial enzymes (NADH dehydrogenase and cytochrome c oxidase) activities. However, rats treated with chrysin (10, 20 and 40 mg kg^{-1}, p.o.) failed to produce any significant inhibition in altered levels of antioxidant, inflammatory, apoptosis, AKI markers and mitochondrial depleted enzymes. Histopathological abbreviations were also did not ameliorates by chrysin treatment. **Conclusion:** Chrysin failed to produce any significant protection against gentamycin-induced renal toxicity.

Key words: Chrysin, gentamicin, kidney injury molecule-1, mitochondrial enzymes, nephrotoxicity neutrophil gelatinase-associated lipocalin, oxidative stress

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Clinically gentamicin is one of the most commonly used aminoglycosides antibiotic for the treatment of life-threatening Gram-negative bacterial infections. However, it is associated with serious nephrotoxicity side effect thus its prescription has been restricted[1]. Around 30% of patients administered with gentamicin suffered from serious side effect such as renal toxicity[2]. Exact mechanism underlying cause of gentamicin-induced nephrotoxicity has not been yet evaluated however, various evidence suggesting that the possible mechanism behind the gentamycin-induced nephrotoxicity are mainly due to generation of Reactive Oxygen Species (ROS), such as superoxide anions, hydroxyl radicles and hydrogen peroxide[3], Reactive Nitrogen Species (RNS)[4], Na^+-K^+-ATPase inhibition and inhibition of mitochondrial oxidative phosphorylation[3-5].

Many experimental animal models have been designed to reproduce clinic-pathological features of chemically-induced nephrotoxicity and to develop new therapeutic moieties for its treatment[6-8]. However, gentamycin-induced nephrotoxicity is well-established and reproducible animal model to evaluate the potential of an array of therapeutic moieties[2,9]. As of late, the quest for proper nephroprotective moieties has been again centered around plants as a result of less toxicity, simple accessibility and simple assimilation in the body that may be preferred treatment over as of synthetic medications. Ayurveda, Unani and Chinese endorse various medications for renal diseases[10,11]. Recently herbal nephroprotective agents have gained significance to fill the lacunae made by the synthetic chemical moieties. Plant products are thought to be less toxic than synthetic medications which are formed by compound amalgamation.

Since ancient times, plants have been commendable wellsprings of medication. Plants still constitute one of the major raw materials in medications for treating different diseases of a human being, despite the fact that there has been enormous development in the field of synthetic chemistry. Saponins, tannins, vital oils, flavonoids, alkaloids and other chemical compounds are the biologically active synthetic substances which have healing properties that have presented in medicinal plants[12]. Plants and various plant derivatives have long been utilized as traditional remedies for the treatment of renal disease in numerous parts of the world. In the Indian traditional system of medicine, there are an array of plants and their products that have been utilized for treatment of renal disease. The potential remedial and preventive advantages of plant-based medications have been the subject of broad studies and numerous common constituents have been unrevealed with noteworthy pharmacologic action, including antioxidant and nephroprotective properties[13,14]. Nutraceuticals as well as functional food that are advantageous to various diseases which may speak to valuable intensifies that can decrease the general health risk impelled by acting various maladies if there should arise an occurrence of disappointment or in circumstances where synthetic chemical moieties can't be utilized[15,16].

Chrysin (5,7-dihydroxyflavone) a plant flavonoid obtained from honey, flowers and bee propolis reported possessing potent antioxidant and anti-inflammatory activity[17]. It contains an array of pharmacological properties, such as anti-diabetogenic[18], anti-inflammatory[19], anticancer[20,21] antihypertensive[22], antiviral[23], anti-oestrogenic[24] and anxiolytic activity[25] have also been recounted. The potential neuroprotective effects of chrysin have been well-studied previously[26]. However, its potential against gentamycin-induced nephrotoxicity has been not yet revealing. Thus, the aim of present investigation was to explore the efficacy of chrysin against gentamycin-induced nephrotoxicity in laboratory rats.

MATERIALS AND METHODS

Drugs and chemicals: Gentamicin and methylprednisolone were obtained from Symed Pharmaceuticals Pvt., Ltd., Hyderabad, India. Chrysin, NADH, cytochrome c, mannitol and bacterial protease were purchased from Sigma-Aldrich Co., St Louis, MO, USA. The 1,1,3,3-tetraethoxypropane, crystalline beef liver catalase, 5,5-dithiobis (2-nitrobenzoic acid) were purchased from S.D. Fine Chemicals, Mumbai, India. Sulphanilamides, naphthylamine diamine HCl and phosphoric acid were obtained from Loba Chemie Pvt., Ltd., Mumbai, India. Creatinine and BUN kits were purchased from Accurex Biomedical Pvt., Ltd., Mumbai, India. Total RNA extraction kit and one-step RT-PCR kit was purchased from MP Biomedicals Private Limited, India.

Experimental animals: Adult male Sprague-Dawley rats (220-250 g) were purchased from the National Institute of Biosciences, Pune (India). They were maintained at 24±1°C with a relative humidity of 45-55% and 12:12 h dark/light cycle. The animals had free access to standard pellet chow (Pranav Agro-industries Ltd., Sangli, India) and water throughout the experimental protocol. All experiments were carried out between 09:00 and 17:00 h. The experimental protocol (CPCSEA/PCL/09/2014-15) was approved by the

Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), government of India on animal experimentation.

Experimental design: Rats were randomly divided into following groups (n = 6) as follows:

Group I: **Normal (N):** Rats were orally treated with distilled water (10 mg kg^{-1}, p.o.) and received an intraperitoneal (i.p.) injection of normal saline daily for 7 consecutive days.

Group II: **Gentamicin control (GM-control):** Rats were orally treated with distilled water (10 mg kg^{-1}, p.o.) and an i.p., injection of GM (120 mg kg^{-1}, i.p.) daily for 7 consecutive days.

Group III: **Gentamicin+chrysin (10 mg kg^{-1}) [GM+CRY (10)]:** Rats were treated daily with both chrysin (10 mg kg^{-1}, p.o.) and GM (120 mg kg^{-1}, i.p.) at an interval of 1 h for 7 consecutive days.

Group IV: **Gentamicin+chrysin (20 mg kg^{-1}) [GM+CRY (20)]:** Rats were treated daily with both chrysin (20 mg kg^{-1}, p.o.) and GM (120 mg kg^{-1}, i.p.) at an interval of 1 h for 7 consecutive days.

Group V: **Gentamicin+chrysin (40 mg kg^{-1}) [GM+CRY (40)]:** Rats were treated daily with both chrysin (40 mg kg^{-1}, p.o.) and GM (120 mg kg^{-1}, i.p.) at an interval of 1 h for 7 consecutive days.

Group VI: **Gentamicin+methylprednisolone (12.5 mg kg^{-1}) [GM+MP (12.5)]:** Rats were treated daily with both methylprednisolone (12.5 mg kg^{-1}, i.p.) and GM (120 mg kg^{-1}, i.p.) at an interval of 1 h for 7 consecutive days.

Nephrotoxicity was induced in rats (except normal) by GM at a dose of 120 mg kg^{-1}, i.p. for 7 days[9]. The GM was dissolved in normal saline. Doses of chrysin (20 and 40 mg kg^{-1}) were selected on the basis of the previous study carried out in this laboratory[26]. The dose of methylprednisolone (12.5 mg kg^{-1}) was selected on the basis of the previous study[27]. At the end of the study, whole blood samples were collected from retro-orbital plexus to obtain serum for renal function parameters (creatinine and BUN). Body weights and kidney weights of all animals were recorded and animals were sacrificed by cervical dislocation. Kidney tissues were harvested, fatty and conjunctive tissue layer were removed, rinsed in normal saline and stored in -80°C freezer for further biochemicals and RT-PCR studies. A kidney of rat from each group was isolated and fixed in 10% formalin solution for histopathological examination.

Serum biochemistry: The serum was separated by centrifugation using eppendorf cryocentrifuge (model No. 5810, Germany), maintained at 4°C and run at a speed of 7000 rpm for 15 min. Serum creatinine and BUN were measured by a spectrophotometer (UV-visible spectrophotometer, V-530, Japan) using reagent kits according to the procedure provided by the manufacturer (Accurex Biomedical Pvt., Ltd., Mumbai, India).

Biochemical estimation

Kidney tissue homogenate preparation, antioxidants, lipid peroxidation (MDA) and NO estimation: A known weight of the kidney tissue homogenates was prepared with 0.1 M tris-HCl buffer (pH 7.4) and supernatant of homogenates was employed to estimate superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (MDA) and Nitric Oxide (NO) as described previously[26,28-33].

Mitochondrial enzymes estimation: Renal mitochondria were isolated and mitochondrial complex (I-IV) activity was measured spectrophotometrically according to previously described method[34-40].

Determination of KIM-1 and NGAL by reverse transcriptase-PCR in kidney: The levels of mRNA were analyzed in renal tissue using a Reverse Transcription (RT)-PCR approach as described previously[26,41-43]. Briefly, single-stranded cDNA was synthesized from 5 µg of total cellular RNA using reverse transcriptase (MP Biomedicals India Private Limited, India) as described previously[26]. Amplification of β-actin served as a control for sample loading and integrity. The primer sequences for KIM-1, NGAL and β-actin were selected according to the previously reported method[44]. The PCR products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. The size of amplicons was confirmed using a 100 bp ladder as a standard size marker. The amplicons were visualized and images were captured using a gel documentation system (Alpha Innotech Inc., San Leandro, CA, USA). Gene expression was assessed by generating densitometry data for band intensities in different

sets of experiments by analyzing the gel images on the image J program (Version 1.33, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) semi-quantitatively. The band intensities were compared with constitutively expressed β-actin. The intensity of mRNAs was standardized against that of the β-actin mRNA from each sample and the results were expressed as PCR-product/β-actin mRNA ratio.

Histological examination: The dissected kidney tissue specimens were fixed in 10% formaldehyde, processed routinely for embedding in paraffin. Sections were stained with hematoxylin-eosin stain and Masson's trichrome stain as described previously[45]. Kidney sections were analyzed qualitatively under a light microscope (40 and 100X) for various histopathological alterations.

Statistical analysis: Data was expressed as Mean±Standard Error Mean (SEM). Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, CA, USA). Data was analyzed by one-way analysis of variance (ANOVA) and Dunnett's tests were applied for post hoc analysis. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of chrysin on GM-induced alterations in relative kidney weight: There was significant ($p<0.01$) increased the relative kidney weight ratio in gentamycin control rats when compared to normal rats. The Co-administration of methylprednisolone (12.5 mg kg^{-1}, p.o.) with gentamicin significantly ($p<0.001$) decreased the relative kidney weight ratio compared to gentamicin administered control rats. However, chrysin (10, 20 and 40 mg kg^{-1}, p.o.) treatment did not show significant changes in relative kidney weight ratio compared to control rats (Table 1).

Effect of chrysin on GM-induced alterations in renal function parameters: The levels of serum renal function parameters such as creatinine and BUN were significantly ($p<0.01$ and $p<0.001$) increased in gentamicin control rats compared to normal rats. Oral administration of methylprednisolone (12.5 mg kg^{-1}, p.o.) to gentamicin-treated rats significantly ($p<0.001$) decreased the serum creatinine and BUN levels compared to gentamicin control rats. Creatinine clearance was also significantly ($p<0.001$) decreased in gentamicin-treated rats compared to normal rats. However, treatment with methylprednisolone (12.5 mg kg^{-1}, p.o.) significantly ($p<0.001$) increased the creatinine clearance compared to gentamicin alone treated rats. However, chrysin (10, 20 and 40 mg kg^{-1}, p.o.) failed to reduce serum creatinine and BUN levels as well as failed to increase creatinine clearance as compared to gentamicin administered control rats (Table 1).

Effect of chrysin on GM-induced alterations in oxidative stress: Renal SOD and GSH levels were significantly ($p<0.05$ and $p<0.01$) decreased in gentamicin alone treated rats when compared to normal rats. The Co-administration of methylprednisolone (12.5 mg kg^{-1}, p.o.) with gentamicin significantly ($p<0.01$ and $p<0.001$) increased the renal SOD and GSH levels compared to gentamicin administered rats. However, chrysin (10, 20 and 40 mg kg^{-1}, p.o.) administration failed to produce a significant effect on the renal SOD and GSH levels in gentamicin administered rats (Table 2).

Table 1: Effect of chrysin on GM-induced alterations in relative kidney weight, serum creatinine, BUN and creatinine clearance in rats

Parameters	Normal	GM-control	GM+CRY (10)	GM+CRY (20)	GM+CRY (40)	GM+MP (12.5)
Relative kidney weight	0.48±0.03	0.70±0.05$^{\#\#}$	0.68±0.03	0.65±0.02	0.64±0.04	0.52±0.06***
Serum creatinine (mg dL^{-1})	0.76±0.21	2.89±0.38$^{\#\#}$	2.92±0.30	2.82±0.38	2.70±0.32	0.81±0.44***
BUN (mg dL^{-1})	22.91±2.21	85.63±3.22$^{\#\#\#}$	82.32±4.21	80.23±4.55	83.74±2.44	36.52±8.22***
Creatinine clearance (mL min^{-1})	22.32±2.11	3.26±0.47$^{\#\#\#}$	4.56±0.84	5.63±0.45	6.66±1.85	16.32±2.96***

Data are expressed as Mean±SEM (n = 5) and analyzed by one-way ANOVA followed by *post hoc* Dunnett's tests. ***p<0.001 as compared with GM-control group and $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 as compared with normal group, CRY: Chrysin, BUN: Blood urea nitrogen, GM: Gentamicin and MP: Methylprednisolone

Table 2: Effect of chrysin on GM-induced alterations in oxido-nitrosative stress in rats

Parameters	Normal	GM-control	GM+CRY (10)	GM+CRY (20)	GM+CRY (40)	GM+MP (12.5)
SOD (U mg^{-1} protein)	12.12±0.95	6.23±0.89$^{\#}$	7.52±0.12	8.55±1.11	8.12±0.37	10.85±0.63**
GSH (mg mg^{-1} protein)	42.56±4.12	20.44±3.12$^{\#\#}$	21.22±2.16	19.41±3.55	22.30±4.12	38.96±2.63***
MDA (nmol L^{-1} mg^{-1} protein)	0.38±0.08	3.66±0.78$^{\#\#}$	3.16±0.52	3.08±0.12	3.52±0.33	0.62±0.18***
NO (μg mL^{-1})	100.62±8.66	201.52±26.32$^{\#\#}$	209.63±11.78	200.16±12.12	192.63±18.20	120.33±21.52***

Data are expressed as Mean±SEM (n = 5) and analyzed by one-way ANOVA followed by *post hoc* Dunnett's tests. **p<0.01, ***p<0.001 as compared with GM-control group and $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 as compared with normal group, CRY: Chrysin, BUN: Blood urea nitrogen, GM: Gentamicin, MP: Methylprednisolone, SOD: Superoxide dismutase, GSH: Reduced glutathione, MDA: Malondialdehyde and NO: Nitric oxide

Effect of chrysin on GM-induced lipid peroxidation and NO alteration: Gentamicin administration produced a significant ($p<0.05$) increase in kidney tissue MDA and NO levels as compared to normal rats. Addition of methylprednisolone (12.5 mg kg^{-1}, p.o.) treatment to gentamicin significantly ($p<0.001$) restored the levels of kidney tissue MDA and NO compared to gentamicin alone treated rats. Furthermore, administration of chrysin (10, 20 and 40 mg kg^{-1}, p.o.) did not show a significant effect on kidney tissue MDA and NO levels compared to gentamicin control rats (Table 2).

Effect of chrysin on GM-induced renal mitochondrial dysfunction: The activities of NADH dehydrogenase (complex-I) and cytochrome c oxidase (complex-IV) were significantly ($p<0.001$) reduced in renal mitochondria isolated from gentamicin-treated control rats when compared to normal rats. Oral administration of methylprednisolone (12.5 mg kg^{-1}, p.o.) along with gentamicin significantly ($p<0.001$) restored the NADH dehydrogenase and cytochrome c oxidase activities compared with gentamicin alone treated group. Chrysin (10, 20 and 40 mg kg^{-1}, p.o.) failed to show significant changes in NADH dehydrogenase and cytochrome c oxidase activities when compared with gentamicin control rats (Fig. 1).

Effect of chrysin on GM-induced alteration in renal KIM-1 and NGAL mRNA expressions: Gentamicin administration produced a significant ($p<0.001$) upregulation in renal KIM-1 and NGAL mRNA expressions as compared to normal rats. Administration of methylprednisolone (12.5 mg kg^{-1}, p.o.) significantly ($p<0.001$) downregulated the levels of renal KIM-1 and NGAL mRNA expressions as compared to gentamicin alone treated rats. When compared with gentamicin control rats, chrysin (10, 20 and 40 mg kg^{-1}, p.o.)

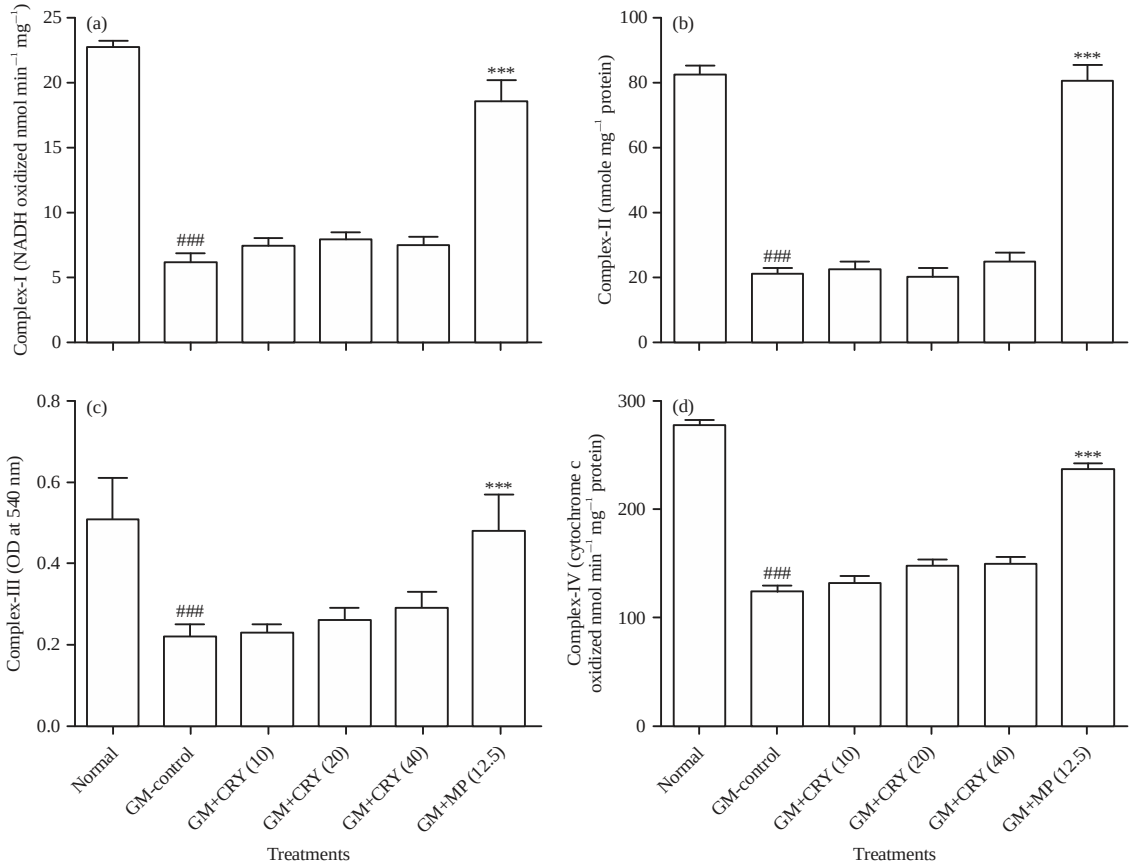

Fig. 1(a-d): Effect of chrysin on GM-induced alterations in renal mitochondrial enzyme activities in rats, (a) Complex-I, (b) Complex-II, (c) Complex-III and (d) Complex-IV. Data are expressed as Mean±SEM (n = 5) and analyzed by one-way ANOVA followed by *post hoc* Dunnett's tests. ***$p<0.001$ as compared with GM-control group and $^{###}p<0.001$ as compared with normal group, CRY: Chrysin, BUN: Blood urea nitrogen, GM: Gentamicin and MP: Methylprednisolone

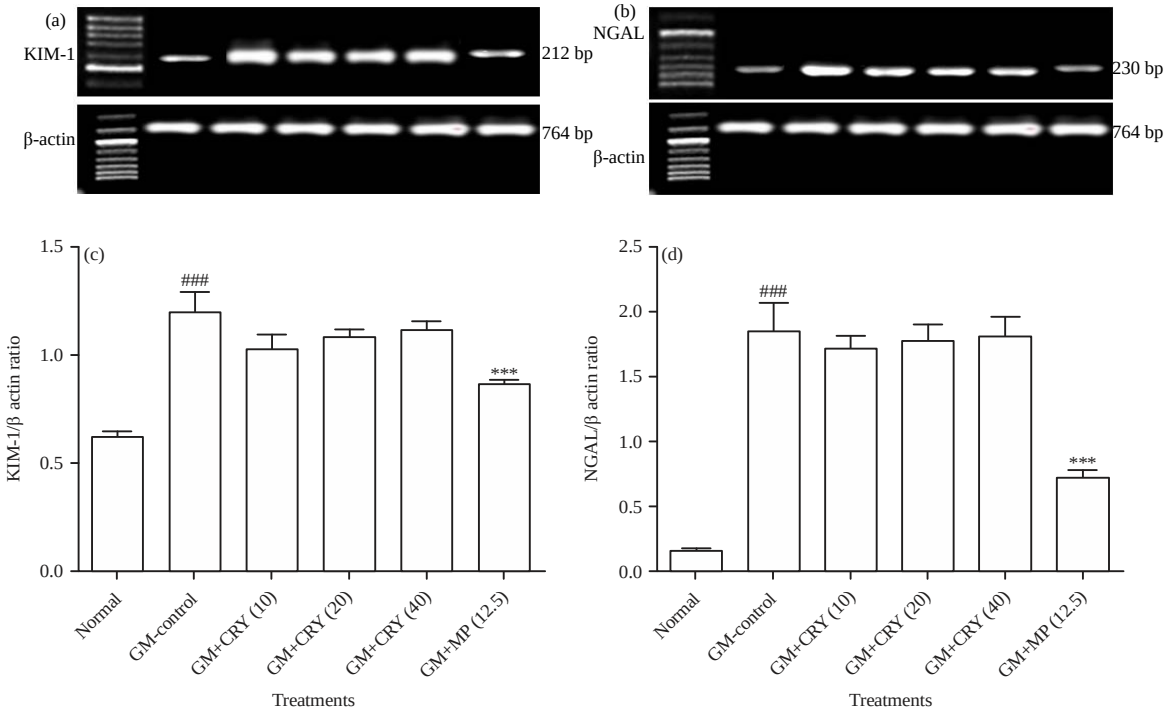

Fig. 2(a-d): Effect of chrysin on GM-induced alterations in renal (a) KIM-1, (b) NGAL, (c) KIM-1/β-action ratio and (d) NGAL/β-action ratio mRNA expression in rats, data are expressed as Mean±SEM (n = 5) and analyzed by one-way ANOVA followed by *post hoc* Dunnett's tests. ***p<0.001 as compared with GM-control group and ###p<0.001 as compared with normal group, CRY: Chrysin, BUN: Blood urea nitrogen, GM: Gentamicin and MP: Methylprednisolone

Table 3: Effect of chrysin on GM-induced alterations in kidney histology in rats

Parameter	Glomerular hypertrophy	Inflammatory infiltration	Congestion	Edema	Necrosis
Normal	0	0	0	0	0
GM-control	++++	+++	++++	+++	++++
GM+CRY (20)	++++	+++	++++	+++	+++
GM+CRY (40)	+++	+++	++++	+++	+++
GM+MP (12.5)	+++	+++	+++	+++	++

0: No abnormality detected, +: Damage/active changes up to less than 25%, ++: Damage/active changes up to less than 50%, +++: Damage/active changes up to less than 75%, ++++: Damage/active changes up to more than 75%

treatment failed to produce any significant inhibition in a gentamicin-induced alteration in renal KIM-1 and NGAL mRNA expressions (Fig. 2).

Effect of chrysin on GM-induced histological alteration in renal tissue: Kidney tissue from normal rat showed intact glomerulus basement membrane and tubules without any congestion, necrosis and inflammatory infiltration (Fig. 3a). Administration of gentamicin resulted in renal damage reflected by the presence of intrinsic lesions (grade 4) within the glomeruli and epithelium, glomerular hypertrophy (grade 4) along with intracellular edema (grade 3) and inflammatory infiltration (grade 3) (Fig. 3b). There was decreased inflammatory infiltration, intracellular edema and necrosis (grade 1) in histology of renal tissue from methylprednisolone (12.5 mg kg^{-1}) treated rats reflected a reduction in gentamicin-induced renal damage (Fig. 3c). Renal tissue from chrysin (40 mg kg^{-1}) treated rats showed the presence of inflammatory infiltration with mild glomerular hypertrophy, intracellular edema and necrosis (Fig. 3d, Table 3).

DISCUSSION

In clinical settings gentamicin is widely used antibiotic for the treatment of various infections caused by Gram-negative aerobes[2]. However, due to its major side effect of nephrotoxicity limits its clinical utility[46,47]. Thus it's a need of the hour to protect gentamicin-induced kidney injury by utilizing various therapeutic approach. It is well accepted that

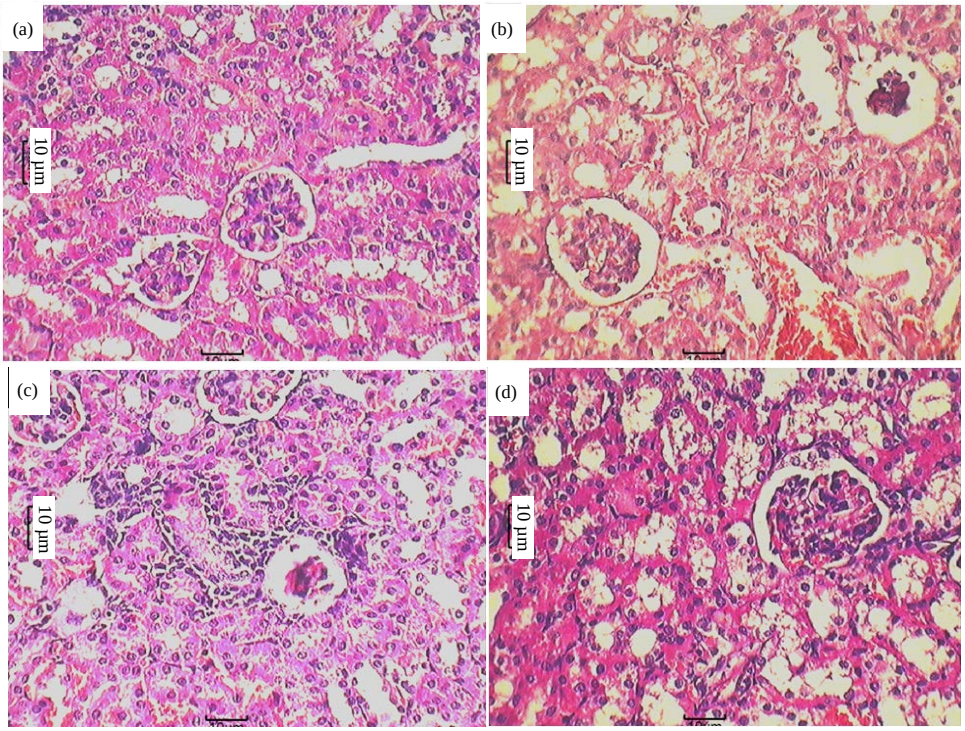

Fig. 3(a-d): Effect of chrysin on GM-induced alterations in kidney histology in rats. Photomicrograph of sections of the kidney of (a) Normal, (b) GM-control rats, (c) Chrysin (80 mg kg^{-1}) treated rats and (d) Methylprednisolone (12.5 mg kg^{-1}) treated rats, H and E staining at 40X

free radical generation is the consequence of reaction of reactive oxygen species resulted in the generation of oxidative stress. In the past, the researchers have underlined the correlation between oxidative stress and gentamicin-induced nephrotoxicity[2,9]. Few recent studies also have suggested that plant flavonoids with antioxidant potential have the ability to inhibit gentamycin-induced nephrotoxicity[9]. Hence, in the present investigation, it have evaluated that the effect of chrysin in gentamicin-induced nephrotoxicity in laboratory animals by assessing various biochemical molecular and histological parameters.

Reactive Oxygen Species (ROS) played a decisive role in the initiation and maintenance of vicious cycle of oxidative stress that caused kidney damage. Activation of inflammatory cells (such as monocytes/macrophages, polymorphonuclear leucocytes (PMNs) and endothelial cells) caused tissue infiltration followed by respiratory burst resulted in increased oxygen utilization which leading to elevated production of various physiological messengers including cytokines (TNF-α, IL-1, IL-6, interferons and colony stimulating factor), nitric oxide, ROS and other mediators of inflammation culminating in inflammation and tissue damage[48-54]. An earlier report suggested that gentamicin elevates the oxidative stress in renal tissue which is a key factor for the development of nephrotoxicity[2,9]. Thus, anti-oxidative agents might be beneficial to overcome the gentamycin-induced nephrotoxicity.

Superoxide dismutase (SOD) is an endogenous enzyme possesses the ability to downregulate oxidative stress via conversion of O^{2-} to H_2O_2 to reduced elevated ROS[55-58]. Whereas glutathione (GSH), a non-enzymatic antioxidant plays a vital role in cellular defense against elevated H_2O_2 generation[35,59-61]. It can decrease cytotoxic H_2O_2 level by its conversion to an oxidized form of glutathione (GSSG) via catalase[62-64]. It has been reported that gentamycin acts as an iron chelator and that the iron-gentamycin complex is a potent catalyst of free radical formation an important causative factor for oxidative stress[65-68]. An earlier report suggested that gentamycin administration spoils the glutathione redox status and decrease the GSH level in the renal tissue[2,9]. In the present study, it has been observed that gentamycin-intoxicated rats demonstrated significantly reduced the activities of SOD and GSH in the renal tissue. However, administration of chrysin failed to inhibit gentamycin-induced decreased SOD and GSH activities in gentamycin-intoxicated rats.

Ferritin delivers free iron molecule upon gentamycin exposure via activation of heme oxygenase and this free iron enhance the ROS formation with the highly threatening hydroxyl radicals through Fenton-type reaction[69-71]. Hydroxyl radicals pierce the membrane barriers and react mutagenically with DNA in the cell nucleus and show the toxic effect to the cells[72]. Gentamycin toxicity resulting in ROS generation causes lipid peroxidation followed by deterioration of membrane lipid bilayer arrangement and increased tissue permeability through the inactivation of membrane-bound enzymes and receptors which is an essential feature of oxidative stress[2,9]. Membrane lipid peroxidation (in terms of MDA) reflects the damage to the cellular structure via destruction of the double bonds in the unsaturated fatty acids and is considered to be a reliable marker of oxidative stress[48,49,73,74]. In the present study, gentamycin ingestion causes lipid peroxidation. However, treatment with chrysin failed to produce any significant attenuates in this increased renal MDA level in gentamycin intoxicated rats.

Erstwhile study has outlined that gentamycin intoxicated rats show a significant elevation in NO level[75]. Nitric Oxide (NO) acts as an endothelial releasing factor in various pathological and physiological processes and its boosting level may be noxious for several organs including kidney[2]. In the present investigation, administration of gentamycin caused a significant elevation in renal NO level. Treatment with chrysin did not show any significant reduction in this elevated levels of renal nitric oxide.

Mitochondria are also known as "Cellular power plant" involves the production of oxidative energy for the cells and play a pivotal role in the oxidative phosphorylation[76-80]. Mitochondria is the most important targeted organelles for the oxidative stress that results in the excess generation of ROS, mitochondrial DNA menace and cellular injury that ultimately cause a decline in renal function[81]. This decrease in renal function was reflected by altered levels of serum creatinine, BUN and creatinine clearance. Studies reveal that gentamycin intoxication demur the mitochondrial antioxidant, mitochondrial membrane potential and disrupt the mitochondrial architecture in the renal tissue[2]. In the present investigation, administration of gentamycin resulted in altered mitochondrial enzyme activity which is according to findings of previous investigators, however, administration of chrysin did not show any significant inhibition in this gentamycin-induced altered mitochondrial enzyme activity.

Gentamicin-induced nephrotoxicity associated with Acute Kidney Injury (AKI) or Acute Renal Failure (ARF)[2]. Kidney injury molecule-1 (KIM-1 or T-cell immunoglobulin and mucin-1 (TIM-1)) and neutrophil gelatinase-associated lipocalin (NGAL, also known as lipocalin-2) are two important hallmarks that has been used clinically for the assessment of AKI or ARF. In present investigation administration of gentamycin significantly increased renal KIM-1 and NGAL mRNA expression in gentamicin-induced nephrotoxicity which is in line with previous findings. This enhanced KIM-1 and NGAL mRNA expression did not significantly down-regulated by chrysin treatment.

CONCLUSION

The results of the present study demonstrate the insignificant nephroprotective activity of chrysin against gentamycin-induced renal toxicity, indicating its ineffectiveness against gentamycin-induced nephrotoxicity.

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