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Thymoquinone Supplementation Protects Against Gentamicin-Induced Nephrotoxicity in Rats

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Abstract: Gentamicin (GM) is a widely used antibacterial antibiotic however its optimal clinical benefit is limited by its serious nephrotoxicity and ototoxicity. The present study was aimed to investigate the influence of Thymoquinone (TQ), a compound isolated from *Nigella sativa* plant with predominant antioxidant property on a rat model of GM-induced nephrotoxicity. Four groups of adult male Wistar Albino rats (n = 10 per group) were treated for 10 consecutive days as follow: group 1; received normal saline and served as normal controls, group 2; received TQ (10 mg/kg/day; orally), group 3; received GM (80 mg/kg/day; intraperitoneal injection), and group 4; concurrently received GM + TQ. At day 11, the animals were sacrificed and the following parameters were evaluated: the levels of serum Creatinine (Cr) and Blood Urea Nitrogen (BUN) as biomarkers of renal function, the renal content of Thiobarbituric Acid Reactive Substances (TBARS) as an index of lipid perioxidation and oxidative stress total Glutathione (GSH) content and Glutathione S-Transferase (GST) activity in renal tissues as indices of antioxidant mechanisms and histopathological examination of kidney specimens. The biochemical results showed that GM administration induced nephrotoxicity associated with significant increases in serum levels of Cr, BUN and in renal content of TBARS with significant reductions in the renal GSH level and GST activity. The histopathological findings supported the presence of seriously injured kidney. However, concomitant administration of TQ efficiently reduced the development of GM nephrotoxicity and its associated biochemical and histopathological features. In conclusion, these data prove that TQ mediates; via, at least in part, its antioxidant property, a marked renoprotective effect against GM-induced nephrotoxicity in rats.

Key words: Thymoquinone, antioxidant, gentamicin, nephrotoxicity, oxidative stress

INTRODUCTION

Over the past decades, *Nigella sativa* (NS) which is an annual herbaceous plant belonging to the botanical family of Ranunculaceae and commonly known as black seed or black cumin has attracted a great deal of attention as a promising folk medicine for various diseases (Randhawa and Alghamdi, 2011). This ancient herb goes by many different names for example in old Latin it is called as 'Panacea' meaning 'cure all' while in Arabic it is termed as 'Habbah Sawda' or 'Habbat el Baraka' translated as Seeds of blessing. In India it is called as Kalonji while in China it is referred as Hak Jung Chou (Aggarwal *et al*., 2008).

Thymoquinone (TQ) has been found as the main bioactive constituent of the volatile oil of NS seeds (Padhye *et al*., 2008). Recently, clinical and experimental studies have demonstrated many therapeutic effects of TQ including immunomodulative (Salem, 2005), anti-inflammatory (Al-Ghamdi, 2001), anti-tumour (Rooney and Ryan, 2005), renoprotective (Badary, 1999), gastroprotective (Kanter *et al*., 2005), cardioprotective, pulmonary protective (Kanter, 2011) and antimicrobial (Harzallah *et al*., 2011) activity. The pivotal antioxidant properties of TQ against oxidative damages induced by a variety of oxidative stress and free radical-generating agents have also been strongly confirmed (Nagi and Mansour, 2000; Kanter *et al*., 2005; Aggarwal *et al*., 2008; Randhawa and Alghamdi, 2011).

Gentamicin (GM) is an aminoglycoside antibiotic commonly used in treating severe and life-threatening infections caused by gram-negative bacteria that are likely to be resistant to most antibacterial agents (Chambers, 2007). Despite its beneficial effect, the clinical use of gentamicin is limited by the fact of its serious nephrotoxicity causing acute renal failure in 10-20% even at its therapeutic levels (Morales *et al*., 2006). The exact underlying pathogenic principals of GM-induced nephrotoxicity still obscure however, some mechanisms have been suggested such as the involvement of lipid peroxidation and oxidative stress with generation of reactive oxygen species (Cuzzocrea *et al*., 2002;

Silan *et al*., 2007). Therefore, the present study was designed to investigate the possible protective effects of TQ against the development and outcome of GM-induced nephrotoxicity in rats.

MATERIALS AND METHODS

The rats were housed in metabolic cages and had free access to tap water and rat chow. Thymoquinone (2-isopropyl-5-methyl-1, 4-benzoquinone) was purchased from Sigma Chemical (St. Louis, MO, USA) and freshly dissolved in physiologic saline on the same day of each using. Gentamicin (80 mg ampoules) was purchased from Pharmaceutical Co. All other chemicals used were of the highest analytical grade.

Animal treatments and experimental design: A total of 40 adult male Wistar Albino rats, weighing 230-260 g were used in this study and housed in metabolic cages with a 12:12 h light-dark cycle at a constant temperature of 23-25°C and had free access to standard laboratory food and water. The animals were divided into four groups (10 rats per group) and treated for 10 consecutive days as follow: group 1; intraperitoneally (i.p.) injected with normal saline (5 mL/kg/day) and served as normal controls, group 2; received oral TQ (10 mg/kg/day) via gastric gavage, group 3; i.p. injected with GM (80 mg/kg/day) and group 4; simultaneously received GM + TQ (by the same aforementioned doses as in groups 2 and 3). The animals were sacrificed at 24 h after the last injection (i.e., at day 11) and their blood samples and kidneys were isolated and prepared for the target studies. Noticeably, the GM administration protocol that was followed in the present study is commonly used experimental model of GM-induced severe nephrotoxicity and acute renal failure (Cuzzocrea *et al*., 2002; Bledsoe *et al*., 2006). In addition, the daily dosage regimen of TQ that was administered to rats is according to the earlier studies in which oral TQ was used effectively and safely (Badary, 1999; Khattab and Nagi, 2007).

Sacrificing and sampling: At the end of the study, the animals were sacrificed under ether anesthesia. Blood samples were drawn from the inferior vena cava and allowed to clot and retract at room temperature for 1 h before centrifugation at 3,000×g for 10 min. Supernatants (i.e., serum samples) were separated and stored at -20°C until used. Both kidneys of each rat were immediately excised, weighed and rinsed with a PBS solution, pH 7.4, to remove any blood cells and clots. The right kidney was used for histological examination while the left one was cut into small pieces, homogenized in 5 volume of ice-cold Tris HCl buffer (50 mM, pH 7.4) and centrifuged at 10,000×g for 10 min. The volume of the resultant supernatant was measured and stored at -20°C until used.

Biochemical analysis of kidney function: The levels of serum Creatinine (Cr) and Blood Urea Nitrogen (BUN) as well-established endogenous biomarkers in estimating kidney function and glomerular filtration rate (were measured using commercial assay kits (BioAssay Systems, Hayward, CA) and following the manufactures' instructions.

Histological examination: Isolated kidney specimens were fixed in 10% neutral formalin, paraffinized and processed for histological examination. Paraffin sections at 4 m thickness were prepared from each kidney, stained with Hematoxylin and Eosin (H&E) and then microscopically examined for the existence of renal tubular and glomerular damage, tubulointerstitial nephritis as well as cellular infiltration.

Assessment of renal lipid peroxidation and antioxidant defense states: Levels of Thiobarbituric Acid Reactive Substances (TBARS) as a representative for the extent of lipid peroxidation as well as Glutathione (GSH) content and the activity of its related enzyme; Glutathione-S-Transferase (GST) as indices of the antioxidant defence state were specrophotometrically assayed in the prepared supernatants of kidney tissue homogenates using commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits (Sigma-Aldrich, St. Louis, Mo., USA) and according to the manufactures' instructions.

Statistical analysis: Statistical analysis was performed using the Statistical Package for Social Science (SPSS) Version 16 (SPSS Inc., Chicago, IL, USA). All data are presented as means±SD. Differences in the various parameters in more than two groups were evaluated by a one-way ANOVA. Continuous variables between two groups were analyzed using Student's t-test. Differences between groups were considered significant at $p<0.05$.

RESULTS

Effects of thymoquinone on gentamicin-induced renal injury in rats: As shown in Table 1, i.p. injections of GM (80 mg/kg/day) into rats for 10 consecutive days resulted in marked impairment of their renal functions as reflected by significant increases in the levels of serum Cr and BUN as compared with values of normal controls. On the other hand, concomitant oral administration of TQ (10 mg/kg/day) into these GM-injected animals produced

Table 1: Effects of Thymoquinone (TQ) and Gentamicin (GM) on kidney functions and renal levels of total Glutathione (GSH), activity of Glutathione S-Transferase (GST) and concentrations of Thiobarbituric Acid-Reactive Substances (TBARS) in rats

	Groups			
Parameters	Control	GM	GM + TQ	TQ
Creatinine (mg dL^{-1})	0.63±0.03	1.31±0.09[a]	0.89±0.08[d, c]	0.64±0.04[c]
BUN (mg dL^{-1})	32.50±1.60	79.40±5.50[a]	39.00±1.70[d, c]	31.70±1.50[c]
GSH content (µmol mL^{-1})	6.37±1.00	1.70±0.30[b]	5.80±0.90[d, c]	7.70±1.10[a]
GST activity (µmol/mL/min)	3.03±0.30	0.85±0.09[b]	2.79±0.08[d, c]	3.84±0.84[a]
TBARS content (µmol mL^{-1})	450.00±27.0	1600.00±103[b]	500.00±66.0[d, c]	380.00±37.0[a]

Values are expressed as the mean±SD (n = 10). [a]p<0.05, [b]p<0.01 and [c]p = NS versus normal control group; [d]p<0.05 and [e]p = NS versus GM alone received group

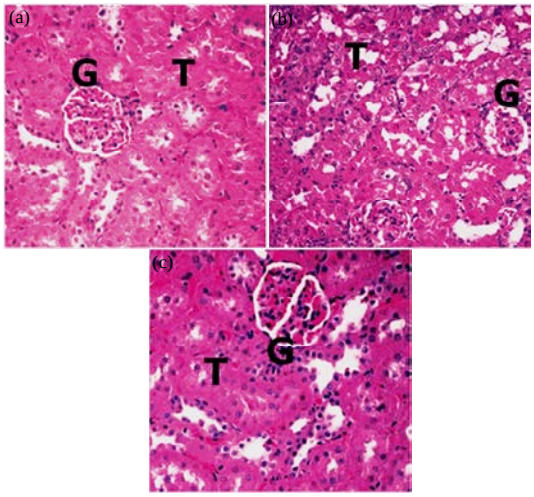

Fig. 1: Histopathological findings: a) Kidneys of normal rats showing normal Glomerular (G) and Tubular (T) histomorphology. b) Kidneys of rats received gentamicin alone; demonstrating clear hallmarks of severely injured necrotic glomeruli and tubular necrosis and interstitial nephritis. c) Kidneys of rats received a combination of Thymoquinone (TQ) plus gentamicin revealing significant attenuating effects of TQ against gentamicin-induced glomerular and tubular injuries

an efficient protecting effect on their kidney functions whereas the levels of serum Cr and BUN were almost reserved to the control values (Table 1).

Effects of thymoquinone on gentamicin-induced lipid peroxidation and depletion of antioxidant defence in rat kidneys: As shown in Table 1, GM, 80 mg/kg/day for 10 successive days produced remarkable increases in the levels of TBARS as well as significant decreases in the level of GSH and the activity of GST in rat renal tissues that collectively indicating that GM administration resulted in induction of lipid peroxidation and oxidative damage in the kidney tissues. By contrast, simultaneous administration of TQ (10 mg/kg/day) significantly reversed these depleting effects of GM on renal GSH and GST and prevented its inducing effect on TBARS (Table 1). More interestingly, when TQ was administered alone into normal rats, it interestingly increased the GSH content and GST activity and also decreased TBARS concentrations in their renal tissues; confirming its antioxidant potentiality.

Histopathological observations: The histopathological findings supported the aforementioned biochemical data and confirmed the remarkable renoprotective effects of TQ against GM-induced nephrotoxicity in rats. As illustrated in Fig. 1, H&E staining of the kidney specimens from control rats revealed normal morphology of renal tubules and glomeruli (Fig. 1a). By contrast, kidney sections of rats received daily GM alone for 10 consecutive days (Fig. 1b) showed clear histopthological hallmarks of severely induced glomerular and tubular degenerative changes and necrosis, interstitial nephritis and sloughing of the tubular epithelial cells. However, simultaneous administration of TQ to GM-injected rats revealed almost a complete preservation of almost normal histomorphology of renal glomeruli and tubules (Fig. 1c).

DISCUSSION

This study indicated that supplementation of thymoquinone mediated a marked renoprotective effect against gentamicin-induced nephrotoxicity in rats.

Gentamicin (GM) is an aminoglycoside antibiotic that has a very useful role in treating many serious infections caused by Gram-negative bacteria, particularly in critically ill patients (Chambers, 2007). Despite its beneficial effects, GM, like other aninoglycosides has a high tendency to produce severe nephrotoxicity that can cause acute renal failure in 10-20% at its therapeutic courses (Morales *et al*., 2006). In view of this importance, the goal of reducing or preventing the development nephrotoxicity associated with GM therapy has attracted considerable efforts. Mechanistically, several studies have been reported that depletion of antioxidant defence and induction of lipid

peroxidation and oxidative stress in renal tissues are the fundamental underlying pathogenic mechanisms of GM-induced nephrotoxicity (Vardi *et al*., 2005; Silan *et al*., 2007). In this regard, it has been suggested that the using of agents with powerful antioxidant properties can interfere with the development of GM-associated nephrotoxicity and renal failure (Velasco-Velazquez *et al*., 2006; Silan *et al*., 2007). Accordingly, the present study was designed to investigate the possible renoprotective effects of Thymoquinone (TQ), a compound derived from the volatile oil of the *Nigella sativa* seeds with well-evidenced antioxidant properties on rat model of GM-induced nephrotoxicity.

In this research, i.p. injections of GM into rats (80 mg/kg/day for 10 successive days) resulted in development of destructive renal injury that was associated with significant elevations in the levels of serum Cr and BUN. In addition, the findings of histopathological examinations confirmed the biochemical data and showed the clear signs of nephrotoxicity in the form of marked glomerular and tubular degenerative changes and necrosis, tubulointerstitial nephritis and dilatation of the tubular lumen. These biochemical and histopathological observations of GM nephrotoxicity run in consistency with those reported earlier in human patients (Baciewicz *et al*., 2003) and experimental animals (Vardi *et al*., 2005; Silan *et al*., 2007). On the other hand, when TQ (10 mg/kg/day; orally) was concurrently administered with GM, it efficiently protected the rat kidneys from the serious nephrotoxic effects of GM whereas the levels of serum Cr and BUN were kept almost to the control values and the histomorphology of the kidneys was entirely normalized. Also, these results support those of the earlier studies that demonstrated the protective properties of TQ against nephrotoxicity induced by other chemotherapeutic agents such as cisplatin, ifosfamide and doxorubicin (Badary, 1999; Sayed, 2008) as well as against hypertension-induced renal damage (Khattab and Nagi, 2007).

In the present study, GM significantly decreased the content of total Glutathione (GSH) and the activity of its related enzyme; Glutathione S-Transferase (GST) in rat renal tissues. Similar observations have been earlier reported by Cuzzocrea *et al*. (2002). In contrast, co-administration of TQ prevented entirely the depleting effects of GM on renal GSH and GST. These results are in agreement with the findings of earlier studies showed the powerful inhibitory effects of TQ against chemicals-induced depletion of GSH and its related antioxidant enzymes (Farah *et al*., 2005; Padhye *et al*., 2008; Sayed, 2008).

It is evident that the content of GSH and the activity of its related enzymes, particularly GST, constitute the essential antioxidant and detoxification elements in most human and animal cells and tissues (Baillie and Slatter, 1991). In addition, the intracellular GSH status and the activity of GST appear to be sensitive indicators of the overall health of a cell and its ability to resist toxic challenges (Baillie and Slatter, 1991). Moreover, Silan *et al*. (2007) showed that kidneys from GM-treated rats have a weak or even no antioxidant defence mechanism and are more vulnerable to reactive oxygen species. Also, Kanter *et al*. (2005) reported that due to induction of GSH and GST in liver tissues, TQ mediated a marked chemopreventive-antioxidant effect in hepatic disorders via induction of GSH and GST in liver tissues. Based on these collective evidences, it could be suggested that the preventative effect of TQ against GM-induced depletion of renal GSH and GST is at least in part, an important event in the renoprotective effects of TQ against GM-induced nephrotoxicity and acute renal faliure.

In a supplemental manner, lipid peroxidation of cell membranes is also considered as one of the major reasons for oxygen radical-induced cellular and tissue injury. Measurement of Thiobarbituric Acid-Reactive Substances (TBARS) is a widely employed assay in determining lipid peroxidation and oxidative damage (Dawan-Linsley *et al*., 2005). In the present study, the concentrations of TBARS in renal tissues of all animal groups were measured and their values showed that GM significantly increased TBARS levels. Thereby, it could be suggested that lipid peroxidation and oxidative stress induced by GM might play a role in GM-induced kidney damage. On the other hand, the combined treatment of TQ with GM clearly prevented these inducing effects of GM on renal TBARS. In support with these results, a strong relationship between GM-nephrotoxicity, lipid peroxidation and oxidative stress has been earlier confirmed (Vardi *et al*., 2005; Velasco-Velazquez *et al*., 2006). Also, it has been illustrated that TQ inhibits lipid peroxidation, decreases cellular oxidative stress and stimulates antioxidant defence capacity (Mansour *et al*., 2002). Taking together, it is interesting to note that the findings of the earlier studies run in a full constancy with our findings in the present study wherein both the inducing effects of TQ on renal GSH and GST and its inhibitory effects on lipid peroxidation have clearly been demonstrated.

CONCLUSION

Results of the present study suggests that concurrent administration of TQ prevents the development of GM-induced severe nephrotoxicity in rats by a mechanism related, at least in part, to its ability to

decrease lipid peroxidation and oxidative stress as well as to preserve the activity of anti-oxidant elements and enzymes in kidney tissues.

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