Thymoquinone Supplementation attenuates Hypertension and Renal Damage in Nitric Oxide deficient Hypertensive Rats

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The present study was undertaken to evaluate the protective effect of thymoquinone (TQ), the main constituent of the volatile oil from *Nigella sativa* seeds, in rats after chronic inhibition of nitric oxide synthesis with N\(^{\omega}\)-nitro-L-arginine methyl esters (L-NAME). Rats were divided randomly into different treatment groups: control, L-NAME, TQ and L-NAME + TQ. Hypertension was induced by 4 weeks administration of L-NAME (50 mg/kg/day p.o.). TQ was administered alone or in combination with L-NAME and continued for 4 weeks. The animals were killed, and the serum and kidney tissues were isolated for the determination of creatinine and glutathione (GSH), respectively. Rats receiving L-NAME showed a progressive increase in systolic blood pressure compared with control rats. Concomitant treatment with TQ (0.5 and 1 mg/kg/day p.o.) reduced the increase in systolic blood pressure induced by L-NAME in a dose dependent manner. Kidney injury was demonstrated by a significant increase in serum creatinine and a decrease in GSH in kidney tissue from L-NAME treated rats. Treatment of rats with TQ decreased the elevated creatinine and increased GSH to normal levels. TQ inhibited the *in vitro* production of superoxide radical in enzymatic and non-enzymatic systems. In conclusion, TQ is effective in protecting rats against L-NAME-induced hypertension and renal damage possibly via antioxidant activity. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: thymoquinone; L-NAME; hypertension; glutathione; creatinine.

INTRODUCTION

Several lines of evidence are accumulating denoting a pivotal role for oxidative stress in the pathogenesis of hypertension. Enhanced activity of reactive oxygen species (ROS) has been demonstrated in patients with various hypertensive disorders (Ding *et al.*, 1998; Vaziri *et al.*, 1999). Increased oxidative stress has been demonstrated in different animal models of hypertension including spontaneously hypertensive rats (SHR) (Schnackenberg *et al.*, 1998), rats with CsA-induced hypertension (Navarro-Antolin *et al.*, 1998), in Dahl salt sensitive hypertensive rats (Atarashi *et al.*, 1997), in rats with lead-induced hypertension as well as in rats with chronic renal failure. The contribution of oxidative stress to the pathogenesis of hypertension is suggested to rely upon inactivation of the NO-dependent vasodilator tone. Furthermore, administration of antioxidants succeeded in improving NO availability and in ameliorating hypertension in lead-induced hypertension (Vaziri *et al.*, 1999), chronic renal failure (Vaziri *et al.*, 1998), as well as spontaneous hypertension (Schnackenberg *et al.*, 1998; Schnackenberg and Wilcox, 1999).

The use of natural products as an alternative to the conventional treatment of various diseases has been on the rise in the past few decades. *Nigella sativa*, a natural herb has long been used as a natural medicine for the treatment of many acute, as well as chronic conditions. These include diabetes, hypertension and dermatological conditions (Ali and Blunden, 2003). In spontaneously hypertensive rats, 2 weeks oral administration of *Nigella sativa* extract (0.6 mL/kg/day) decreased the arterial pressure accompanied by increased diuresis (Zaoui *et al.*, 2000).

Thymoquinone (Fig. 1) is the main constituent of the volatile oil from *Nigella sativa* seeds (Houghton *et al.*, 1995). Little is known about the effect of thymoquinone (TQ) on blood pressure especially in hypertensive rats. Acute intravenous administration of TQ (0.2–1.6 mg/kg), or *Nigella sativa* volatile oil, to

![Figure 1. Chemical structure of thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone).](image-url)
normotensive rats decreased the arterial blood pressure and the heart rate in a dose-dependent manner (El Tahir et al., 1993). TQ is reported to possess a strong antioxidant property (Houghton et al., 1995). Previous studies from our laboratory showed that pretreatment with thymoquinone protected organs against oxidative damage induced by a variety of free radical generating agents including doxorubicin-induced cardiotoxicity (Nagi and Mansour, 2000), carbon tetrachloride evoked hepatotoxicity (Nagi et al., 1999) and nephropathy produced by cisplatin (Badary et al., 1997) where oxidative stress is a common denominator of these models of toxicity. Evidence is accumulating denoting a pivotal role for oxidative stress in the pathogenesis of hypertension. Therefore the objective of the present study was to evaluate the possible protective effect of thymoquinone against L-NAME-induced hypertension and renal damage.

MATERIALS AND METHODS

Chemicals. Thymoquinone and L-NAME were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of the highest analytical grades commercially available.

Animals. Male Wistar albino rats, weighing 230–250 g, were obtained from the Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia and were housed in metabolic cages under controlled environmental conditions (25 °C and a 12 h light/dark cycle). Animals had free access to standard rat pellet food and tap water. The protocol of this study was approved by the Research Ethics Committee of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Experimental design. A total of 48 male Wistar albino rats were used and divided at random into six groups of eight animals each. Control, TQ (two groups), L-NAME and L-NAME + TQ (two groups). Hypertension was induced by 4 weeks administration of L-NAME in drinking water at a concentration of 500 mg/L, to account for a daily intake of 50 mg/kg (Baylis et al., 1999) and nephropathy caused by cisplatin (Badary et al., 1997) where oxidative stress is a common denominator of these models of toxicity. Evidence is accumulating denoting a pivotal role for oxidative stress in the pathogenesis of hypertension. Therefore the objective of the present study was to evaluate the possible protective effect of thymoquinone against L-NAME-induced hypertension and renal damage.

RESULTS

Rats receiving L-NAME showed a progressive increase in systolic blood pressure compared with the control rats. This increase was already significant after the second week and reached approximately 50 mmHg at the end of the 4 weeks of treatment (Fig. 2). Concomitant treatment with TQ (0.5 and 1 mg/kg/day p.o.) reduced the increase in systolic blood pressure induced by L-NAME in a dose dependent manner (Figs 2 and 3). The reduction was significant after the third and fourth weeks. No significant effect of TQ from control rats was observed when administered for 4 weeks at 0.5 and 1 mg/kg/day (Fig. 3).

Table 1 shows the effects of L-NAME on serum creatinine in normal and TQ supplemented rats. Administration of L-NAME (50 mg/kg/day) for 4

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successive weeks resulted in a highly significant 153% increase in serum creatinine, while oral administration of TQ alone for 4 successive weeks showed a non-significant change. Interestingly, oral supplementation of TQ to L-NAME-treated rats for 4 successive weeks resulted in a complete reversal of the L-NAME-induced increase in serum creatinine to the control values.

The effects of L-NAME on the GSH content in kidney tissues from normal and TQ supplemented rats are shown in Table 1. L-NAME resulted in a significant 47% decrease in GSH, compared with the control group. Oral supplementation of TQ in combination with L-NAME resulted in a complete reversal of the L-NAME-induced decrease in GSH to the control values.

Table 1 shows the effects of L-NAME, TQ, and their combination on the body weights of rats. L-NAME treatment resulted in a significant 18% decrease in body weight. Concomitant administration of TQ plus L-NAME produced a marked normalization of body weight to the control values.

Thymoquinone inhibited the in vitro enzymatic (xanthine-xanthine oxidase method) and non-enzymatic (phenazine methosulphate method) systems. The IC_{50} for TQ were found to be 0.1 and 1.2 µM and the IC_{50} for quercetin were found to be 1.5 and 7.5 µM, respectively (Table 2).

The study demonstrated the antihypertensive effect of TQ in NO deficient rats in response to chronic L-NAME treatment. The antihypertensive effect of TQ was dose-dependent (0.5 and 1 mg/kg/day).

The antioxidant activities of TQ have been reported previously (Houghton et al., 1995; Kruk et al., 2000).
Reactive oxygen species have been shown to be critical determinants in hypertension (Suzuki et al., 1995). Furthermore, long-term NO inhibition has been shown to be associated with increased vascular superoxide and angiotensin converting enzyme activity in an antioxidan
t-sensitive manner (Usui et al., 1999). In the past couple of years compelling evidence has begun to accumulate denoting the key role of superoxide anion in hyperten
sion pathophysiology and pharmacotherapy. Basal superoxide anion production increased in blood vessels of SHR in comparison with normotensive rats (Nabha et al., 2005). Superoxide anion scavenging by the use of the SOD mimetic, tempol, was shown to lower blood pressure in NO deficient hypertensive rats (Sainz et al., 2005). The SOD mimetic tempol lowered blood pressure increments in other animal models of hyper
tension as well, including SHR (Welch et al., 2005), hyperthyroid hypertensive rats (Moreno et al., 2005) and Dahl salt-sensitive rats (Hisaki et al., 2005). Acute administration of another SOD mimetic, namely M40403, reduced blood pressure back to normal values and restored deranged endothelium-dependent relaxa
tion in SHR (Cuzzocrea et al., 2004). Treatment of Dahl salt-sensitive hypertensive rats with antioxidant vitamins C and E decreased arterial blood pressure in parallel with a reduction of renal superoxide anion production (Tian et al., 2005). On the other hand, superoxide anion supplementation, together with NO, by the use of molsidomine caused a further increase in blood pressure in SHR but decreased it in normotensive rats (Fortepiani and Reckelhoff, 2005). In the present investiga
tion TQ proved to be a potent superoxide anion scavenger possessing a low IC50 (0.1–1.2 μM range) com
pared with quercetin (1.5–7.5 μM range). It is suggested that such superoxide anion scavenging activity is in
volved in the antihypertensive effect of TQ. Consistent with our suggestion, the flavonoid quercetin which is
known to be a scavenger of superoxide anion (Robak and Grydelwski, 1988) attenuated hypertension in chronic nitric oxide deficient rats (Duarte et al., 2002).

In the present study treatment with TQ ameliorated the NO deficiency induced renal damage reflected by the rise in serum creatinine. TQ amelioration of renal
damage was in parallel to the repletion of renal GSH content depleted by chronic L-NAME. Renal depletion of GSH has been shown to be a marker of L-NAME hyperten
sion (Khattab et al., 2005). Treatment of Dahl salt-sensitive hypertensive rats with the SOD mimetic, tempol (Hisaki et al., 2005) or the antioxidant vitamins C and E (Tian et al., 2005) decreased renal damage, including increased serum creatinine, which paralleled hypertension. A central role is assigned to superoxide anion in many animal models of experimental hyper
tension as well as to essential hypertension in humans. Superoxide anion activity was enhanced in L-NAME induced hypertension in correlation with the progres
sion of hypertension and renal deficiency (Kopkan and Majid, 2005, 2006). It may be proposed that TQ renal protective effect is mediated, at least in part, through preservation of a normal redox GSH environment in kidney tissues as well as its superoxide anion scaveng
ing activity demonstrated in vitro in the present investi
gation. The antioxidant activity of TQ was shown to be implicated in the amelioration of drug-induced organ damage. The results are in agreement with previ
ous observations that thymoquinone protects against doxorubicin-induced cardiotoxicity (Nagi and Mansour, 2000), and cisplatin-induced nephrotoxicity (Badary et al., 1997), carbon tetrachloride-induced hepatotoxicity (Nagi et al., 1999) where oxidative stress is a common denominator of these models of toxicity.

In summary, oral supplementation of TQ protected rats from L-NAME-induced hypertension by a mecha
nism related, at least in part, to its ability to scavenge superoxide. TQ possesses a potent inhibitory effect on platelet aggregation (Enomoto et al., 2001). A safe efficacious antihypertensive drug with additional pro
tective effects against platelet aggregation, one of the important cardiovascular risk factors, deserves the effort of thorough and serious research.

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