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# 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

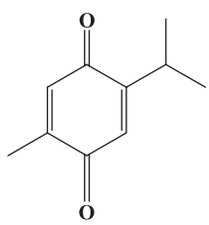

**Figure 1.** Chemical structure of thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone).

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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.*, 1992; Ribeiro *et al.*, 1992). TQ was added either alone (two groups) or in combination with L-NAME (two groups) to drinking water at a concentration of 5 and 10 mg/L, to account for a daily intake of 0.5 and 1 mg/kg, respectively. A control group was also used but without any treatment (drinking water only) for comparison.

**Non-invasive blood pressure monitoring.** Systolic blood pressure was monitored from the tail of each rat every week using the Muromachi BP-Monitor MK-2000 (Muromachi Kikai Co. Ltd, Tokyo, Japan). Briefly, conscious rats were placed on a restrainer of the appropriate size and allowed to equilibrate for a few minutes. The rat tails were placed inside a tail cuff, and the cuff was inflated for systolic blood pressure measurements. The measurements were monitored on a 9 inch display monitor and recorded on a built-in recorder. The average, of at least three consecutive measurements, was taken for presentation.

**Determination of serum creatinine.** Serum creatinine concentrations were measured spectrophotometrically according to the method of Fabiny and Ertingshausen (1971).

**Determination of reduced glutathione in kidney tissues.** The tissue levels of the acid soluble thiols, mainly GSH, were assayed spectrophotometrically at 412 nm, according to the method of Ellman (1959), using a Shimadzu (Tokyo, Japan) spectrophotometer. The contents of GSH were expressed as μmol/g wet tissue.

**Superoxide radical scavenger activity of thymoquinone: xanthine-xanthine oxidase method.** Xanthine-xanthine oxidase was used to generate superoxide radical (Fridovich, 1970) and 2(4-iodophenyl-3-(4-nitrophenol)-5-phenyltetrazolium (INT) chloride was employed for its detection. The reaction mixture contained, in a total volume of 1 mL, 50 mM Tris buffer, pH 10, containing 50 mM xanthine and 25 mM INT. The reaction was started by the addition of 0.4 units of xanthine oxidase. The change in absorbance per minute at 505 nm served as a control reading and was referred to as 100%. TQ or quercetin was added at different concentrations and the measurements were carried out as above. Data were plotted as % inhibition against log TQ or quercetin concentrations and the $IC_{50}$ was obtained from the curve.

**Phenazine methosulphate method.** The reaction mixture contained (Nikishimi *et al.*, 1972) in a total volume of 1 mL, 25 mM Tris-HCl, pH 8.8, 50 mM nitroblue tetrazolium, 100 μM NADH and different concentrations of TQ or quercetin. The reaction was initiated by adding 5 μM phenazine methosulphate and the production of the blue formazan was followed at 560 nm at ambient temperature for 5 min. Data were plotted as % inhibition against log TQ or quercetin concentrations and the $IC_{50}$ was obtained from the curve.

**Statistical analysis.** Differences between obtained values (mean ± SE, $n = 10$) were carried out by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. A value of $p$ of 0.05 or less was taken as a criterion for a statistically significant difference.

## 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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**Table 1. Effect of thymoquinone, L-NAME and their combination on glutathione, creatinine and body weight**

| Treatment | Kidney GSH (μmol/g tissue) | Serum creatinine (mg/dL) | Body weight (g) |
|---|---|---|---|
| Control | 2.88 ± 0.59 | 0.75 ± 0.09 | 299 ± 29 |
| TQ | 2.98 ± 0.22 | 0.81 ± 0.07 | 329 ± 19 |
| L-NAME | 1.52 ± 0.15[a] | 1.90 ± 0.54[b] | 248 ± 33[c] |
| L-NAME + TQ | 2.90 ± 0.28 | 0.85 ± 0.14 | 315 ± 24 |

Data are presented as mean ± SEM ($n$ = 8).
[a, b, c] Significant change from control, at $p < 0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test.

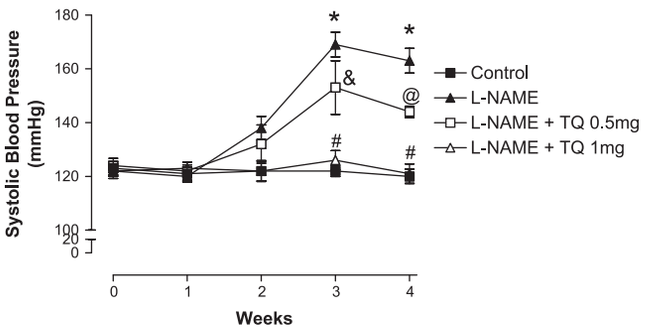


**Figure 2.** Time course on the effect of thymoquinone on L-NAME-induced hypertension in rats. Rats were divided randomly into four different groups of eight animals each: control, TQ, L-NAME and L-NAME + TQ. Hypertension was induced by administration of L-NAME in drinking water at a concentration of 500 mg/L, to account for a daily intake of 50 mg/kg. TQ was added to drinking water at a concentration of 0.5 and 1 mg/L, to account for a daily intake of 0.5 and 1 mg/kg. A control group was also used but without any treatment (drinking water only) for comparison. Results are expressed as mean ± SD of eight rats and data were analysed by one-way ANOVA followed by Tukey-Kramer multiple comparisons test.
* Significant when compared with control at $p < 0.001$.
# Significant when compared with L-NAME at $p < 0.001$.
& and @ Significant when compared with L-NAME at $p < 0.05$ and 0.01, respectively.

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).

**Table 2. Mean $IC_{50}$ values of thymoquinone and quercetin as superoxide radical scavenger using enzymatic and non-enzymatic methods**

| Method | $IC_{50}$ (μM) TQ | Quercetin |
|---|---|---|
| Xanthine-xanthine oxidase | 0.1 | 1.5 |
| Phenazine methosulphate method | 1.2 | 7.5 |

Each value represents the mean of three tests. Standard deviations were less than 10% of the mean.

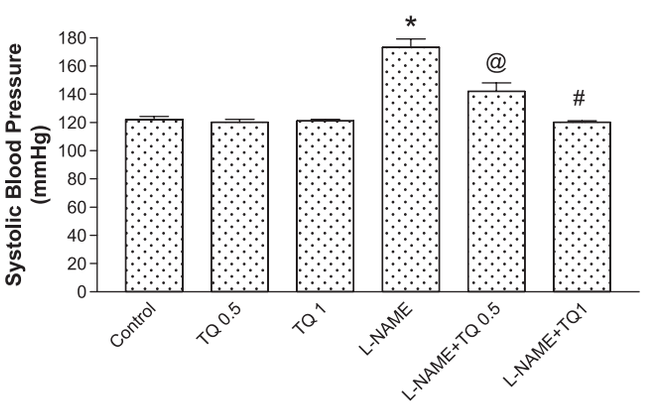


**Figure 3.** Effect of thymoquinone on L-NAME-induced hypertension in rats after 4 weeks. Rats were divided randomly into six different groups of 8 animals each: control, TQ, L-NAME and L-NAME + TQ. Hypertension was induced by administration of L-NAME in drinking water at a concentration of 500 mg/L, to account for a daily intake of 50 mg/kg. TQ was added to drinking water at a concentration of 5 and 10 mg/L, to account for a daily intake of 0.5 and 1 mg/kg/day. A control group was also used but without any treatment (drinking water only) for comparison. Systolic blood pressure was measured. Results are expressed as mean ± SD of 8 rats and data were analysed by one-way ANOVA followed by Tukey-Kramer multiple comparisons test.
* Significant difference from control and TQ (0.5 and 1 mg/kg/day) at $p < 0.001$.
@ Significant difference from L-NAME at $p < 0.01$.
# Significant difference from L-NAME at $p < 0.001$.

## DISCUSSION

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).



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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 antioxidant-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 hypertension 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 hypertension 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 relaxation 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 investigation TQ proved to be a potent superoxide anion scavenger possessing a low $IC_{50}$ (0.1–1.2 μM range) compared with quercetin (1.5–7.5 μM range). It is suggested that such superoxide anion scavenging activity is involved 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 Grydlewski, 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 hypertension (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 hypertension as well as to essential hypertension in humans. Superoxide anion activity was enhanced in L-NAME induced hypertension in correlation with the progression 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 scavenging activity demonstrated *in vitro* in the present investigation. The antioxidant activity of TQ was shown to be implicated in the amelioration of drug-induced organ damage. The results are in agreement with previous 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 mechanism 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 protective effects against platelet aggregation, one of the important cardiovascular risk factors, deserves the effort of thorough and serious research.

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## REFERENCES

Ali B, Blunden G. 2003. Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res* **17**: 299–305.

Atarashi K, Ishiyama A, Takagi M *et al.* 1997. Vitamin E ameliorates the renal injury of Dahl salt sensitive rats. *Am J Hypertens* **10**: 116S–119S.

Badary O, Nagi MN, Al-shabanah O, Al-Sawaf H, Al-Sohaibani M, Al-bekairi A. 1997. Thymoquinone ameliorates the nephrotoxicity induced by cisplatin in rodents and potentiates its antitumor activity. *Can J Physiol Pharmacol* **75**: 1356–1361.

Baylis C, Mitruka B, Deng A. 1992. Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest* **90**: 278–281.

Cuzzocrea S, Mazzon E, Dugo L, Di Paola R, Caputi AP, Salvemini D. 2004. Superoxide: a key player in hypertension. *FASEB J* **18**: 94–101.

Ding Y, Vaziri ND, Gonick HC. 1998. Lead-induced hypertension, II: response to sequential infusions of L-arginine, superoxide dismutase, and nitroprusside. *Environ Res* **76**: 107–113.

Duarte J, Jimenez R, O'Valle F *et al.* 2002. Protective effects of the flavonoid quercetin in chronic nitric oxide deficient rats. *J Hypertens* **20**: 1843–1854.

Ellman GL. 1959. Tissue sulfahydryl groups. *Arch Biochem Biophys* **82**: 70–77.

El-Tahir KE, Ashour MM, al-Harbi MM. 1993. The cardiovascular actions of the volatile oil of the black seed (*Nigella sativa*) in rats: elucidation of the mechanism of action. *Gen Pharmacol* **24**: 1123–1131.

Enomoto S, Asano R, Iwahori Y *et al.* 2001. Hematological studies on black cumin oil from the seeds of *Nigella sativa* L. *Biol Pharm Bull* **24**: 307–310.

Fabiny DL, Ertingshausen G. 1971. Automated reaction-rate method for determination of serum creatinine with the Centrifichem. *Clin Chem* **17**: 696–700.

Fortepiani LA, Reckelhoff JF. 2005. Increasing oxidative stress with molsidomine increases blood pressure in genetically hypertensive rats but not normotensive controls. *Am J Physiol Regul Integr Comp Physiol* **289**: R763–R770.

Fridovich I. 1970. Quantitative aspects of the production of superoxide anion radical by milk xanthine oxidase. *J Biol Chem* **245**: 4053–4057.

Hisaki R, Fujita H, Saito F, Kushiro T. 2005. Tempol attenuates the development of hypertensive renal injury in Dahl salt-sensitive rats. *Am J Hypertens* **18**: 707–713.

Houghton PJ, Zarka R, de las Heras B, Hoult JR. 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med* **61**: 33–36.

Khattab MM, Mostafa A, Al-Shabanah O. 2005. Effects of captopril on cardiac and renal damage, and metabolic alterations in the nitric oxide deficient hypertensive rat. *Kidney Blood Pressure Res* **28**: 243–250.

Kopkan L, Majid DS. 2005. Superoxide contributes to development of salt sensitivity and hypertension induced by nitric oxide deficiency. *Hypertension* **46**: 1026–1031.

Kopkan L, Majid DS. 2006. Enhanced superoxide activity modulates renal function in NO-deficient hypertensive rats. *Hypertension* **47**: 568–572.

Kruk I, Michalska T, Lichszteld K, Kladna A, Aboul-Enein HY. 2000. The effect of thymol and its derivatives on reactions generating reactive oxygen species. *Chemosphere* **41**: 1059–1064.

Moreno JM, Rodriguez Gomez I, Wangensteen R, Osuna A, Bueno P, Vargas F. 2005. Cardiac and renal antioxidant enzymes and effects of tempol in hyperthyroid rats. *Am J Physiol Endocrinol Metab* **289**: E776–E783.

Nabha L, Garbern JC, Buller CL, Charpie JR. 2005. Vascular oxidative stress precedes high blood pressure in spontaneously hypertensive rats. *Clin Exp Hypertens* **27**: 71–82.

Nagi MN, Alam K, Badary OA, Al-Shabanah OA, Al-Sawaf HA, Al-Bekairi AM. 1999. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via antioxidant mechanism. *Biochem Mol Biol Int* **47**: 153–159.

Nagi MN, Mansour M. 2000. Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: a possible mechanism of protection. *Pharmacol Res* **41**: 283–289.

Navarro-Antolin J, Hernandez-Perera O, Lopez-Ongel S, Rodriguez-Puyol D, Lamas S. 1998. CsA and FK506 upregulates eNOS expression: role of reactive oxygen species and AP-1. *Kidney Int* **68**(Suppl): S20–S24.

Nishikimi M, Appaji N, Yagi K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun* **46**: 849–854.

Ribeiro MO, Antanes E, de Nucci G, Lovisolo SM, Zatz R. 1992. Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. *Hypertension* **20**: 298–303.

Robak J, Gryglewski RJ. 1988. Flavonoids are scavengers of superoxide anions. *Biochem Pharmacol* **37**: 837–841.

Sainz J, Wangensteen R, Rodriguez Gomez I *et al.* 2005. Antioxidant enzymes and effects of tempol on the development of hypertension induced by nitric oxide inhibition. *Am J Hypertens* **18**: 871–877.

Schnackenberg CG, Welch WJ, Wilcox CS. 1998. Normalisation of blood pressure and renal vascular resistance in SHR with a membrane-permeable superoxide dismutase mimetic: role of nitric oxide. *Hypertension* **32**: 59–64.

Schnackenberg CG, Wilcox CS. 1999. Two-week administration of tempol attenuates both hypertension and renal excretion of 8-Iso prostaglandin $F_{2\alpha}$. *Hypertension* **33**: 424–428.

Suzuki H, Swei A, Zweifach BW, Schmid-Schonbein GW. 1995. *In vivo* evidence for microvascular oxidative stress in spontaneously hypertensive rats. *Hypertension* **25**: 1083–1089.

Tian N, Thrasher KD, Gundy PD, Hughson MD, Manning RD. 2005. Antioxidant treatment prevents renal damage and dysfunction and reduces arterial pressure in salt-sensitive hypertension. *Hypertension* **45**: 934–939.

Usui M, Egashira K, Kitamoto S *et al.* 1999. Pathogenic role of oxidative stress in vascular angiotensin-converting enzyme activation in long-term blockade of nitric oxide synthesis in rats. *Hypertension* **34**: 546–551.

Vaziri ND, Liang K, Ding Y. 1999. Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. *Kidney Int* **56**: 1492–1498.

Vaziri ND, Oveisi F, Ding Y. 1998. Role of increased oxygen free radical activity in the pathogenesis of uremic hypertension. *Kidney Int* **53**: 1748–1754.

Welch WJ, Mendonca M, Blau J *et al.* 2005. Antihypertensive response to prolonged tempol in the spontaneously hypertensive rat. *Kidney Int* **68**: 179–187.

Zaoui A, Cherrah Y, Lacaille-Dubois MA, Settaf A, Amarouch H, Hassar M. 2000. Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat. *Therapie* **55**: 379–382.




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