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Renal protective effects of thymoquinone against sodium nitrite-induced chronic toxicity in rats: Impact on inflammation and apoptosis

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ABSTRACT

Aims: Sodium nitrite is a widely used color fixative and preservative. However, it has been reported to exert deleterious toxic effects on various body organs. Moreover, thymoquinone (TQ), the active constituent of *Nigella sativa* oil is known to possess beneficial antioxidant and anti-inflammatory effects. The present study was conducted to evaluate the potential protective effects of TQ against sodium nitrite-induced renal toxicity.
Main methods: Male Sprague-Dawley rats were treated with sodium nitrite (80 mg/kg, po, daily) in presence or absence of TQ (25 and 50 mg/kg, po, daily). Morphological changes in renal sections were assessed by staining with Hematoxylin/Eosin and Periodic acid–Schiff. Renal homogenate was used for measurement of oxidative stress markers (MDA and GSH), inflammatory markers (CRP, TNF-α, IL-6, IL-1β), anti-inflammatory cytokines (IL-10 and IL-4) and apoptotic markers (caspase-3/caspase-8/caspase-9).
Key findings: Treatment with sodium nitrite significantly increased markers of renal dysfunction, oxidative stress, inflammation and apoptosis. These effects were markedly attenuated by TQ in dose dependent manner.
Significance: TQ has a potential protective effect against sodium nitrite-induced renal toxicity. This can be attributed to its ability to dampen oxidative stress, restore the normal balance between pro- and anti-inflammatory cytokines and protect renal tissue form extrinsic and intrinsic apoptosis.

1. Introduction

Sodium nitrite is a food additive commonly used in the industry of meat processing as flavor enhancer, color fixative and preservative to prevent growth of several organisms; in particular *Clostridium botulinum* [1]. Moreover, sodium nitrite acts as free radical chelating agent to prevent oxidative rancidity [2]. Further, it is used for industrial production of nitroso compound, dyes and rubber chemicals. At low concentration, sodium nitrite plays important physiological role in human body, as it acts a precursor of nitric oxide. Indeed it is used medically as vasodilator, bronchodilator and for treatment of cyanide poisoning [3]. However, elevated levels have serious toxic effects that include cytotoxicity, embryotoxic and teratotoxicity [4].

Adverse health problems associated with exposure to high quantities of nitrite, especially on infants and children [5]are attributed to its conversion to nitrosamines in overcooked or charred meat or from the production of secondary amines by the acidic stomach [6]. A large body of evidence linked exposure to large quantities of nitrite to development of cancer including brain and gastrointestinal cancers [7], inflammation [8] and various organs toxicity [1,9]. Moreover, ingestion of food rich with sodium nitrite may cause methemoglobinemia, a condition characterized by inhibition of oxygen transport with respiratory problems that may be fatal. Therefore, due to its wide application, studying the biological effects of sodium nitrite is of great demand. Ansari et al. [4] reported that sodium nitrite induced oxidative damage of human erythrocytes with concomitant methemoglobin formation and erythrocyte membrane damage. Due to its wide application and the reported alarming increase in exposure to sodium nitrite beyond non-toxic levels, studying the biological effects of sodium nitrite is of great demand.

Recent therapeutic strategies target natural products in human diets as alternative approaches to prevent development of serious diseases and attenuate body toxicity. Thymoquinone (TQ) is an essential pharmacologically active compound of *Nigella sativa*, which is used for over than 2000 years as popular remedy. TQ, (2-isopropyl-5-methyl-1,4-benzoquinone) constitute about 30–48% of *Nigella sativa* seeds [10]. TQ has been shown to possess wide spectrum of activities including anticancer, antioxidant, anti-inflammatory, anti-hyperglycemic and antibacterial [11–13].

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We had previously reported sodium nitrite-induced toxic injury in various organs [5,9,14,15]. In addition, we recently investigated the curative effects of *Nigella sativa* oil against sodium nitrite-induced nephrotoxicity [14]. Herein, we conducted this study to evaluate if TQ can play a protective role against sodium nitrite-induced nephrotoxicity and to investigate the possible involved mechanisms.

2. Materials and methods

2.1. Experimental design

All experimental procedures adhered to a protocol approved by the ethical committee in Faculty of Pharmacy, Mansoura University. Male Sprague–Dawley rats weighing (120–140 g) were treated with sodium nitrite dissolved in phosphate buffer solution, pH 7.4, once daily early in the morning by oral gavage for 3 months. Rats were assigned into five groups ($n = 10$ for each group).

2.1.1. Control group

Animals received phosphate buffer solution only (3 months, daily) and served as negative control group during the whole study.

2.1.2. TQ treated normal control group

Animals received 50 mg TQ (Sigma-Aldrich, St Louis, MO, USA) dissolved in phosphate buffer solution, pH 7.4, daily for 3 months.

2.1.3. Sodium nitrite-treated group

Animals were treated with daily sodium nitrite (80 mg/kg, orally) for 3 months.

2.1.4. Sodium nitrite + TQ (25 mg/kg) group

Animals received daily TQ (25 mg/kg, orally) followed by sodium nitrite for 3 months.

2.1.5. Sodium nitrite + TQ (50 mg/kg) − group

Animals received daily TQ (50 mg/kg, orally) followed by sodium nitrite for 3 months.

The course of treatment and the doses used for both sodium nitrite and TQ were chosen in accordance to those applied in other studies [10,16–19].

At the end of the experiment, rats were sacrificed. Blood samples were collected and centrifuged at 3000 rpm to separate serum. Thereafter, serum was stored at − 80 °C for further analysis. Rat kidneys were separated, cleaned and homogenized in 10 fold volume of ice-cold sodium potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl then centrifuged at 3000 rpm for 10 min. The supernatants were then stored at − 80 °C.

2.2. Measurement of kidney function tests

Serum levels of creatinine and blood urea nitrogen (BUN) were assessed using commercially available kits (Biodiagnostic Company, Cairo, Egypt) according to manufacturer's instruction.

2.3. Measurement of oxidative stress

Protein content in renal tissue was measured using Bradford method [20]. Then, malondialdehyde (MDA) levels in rat renal tissue were measured by a kit purchased from Biodiagnostic Company (Cairo, Egypt) according to manufacturer's instruction. Renal reduced glutathione (GSH) concentrations were measured as described previously [21]. The principle of the assay based on reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) by glutathione resulting in intense yellow colored product that could be measured at 412 nm.

2.4. Measurement of pro-inflammatory and anti-inflammatory markers by enzyme linked immunosorbent assays (ELISA)

ELISA method was used to measure renal levels of c-reactive protein (CRP), tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β) and IL-6 as well as IL-4 and IL-10 using rat specific ELISA kits purchased form (eBioscience, Inc., San Diego, CA,USA).

2.5. Estimation of activity of caspase-8, caspase 9 and caspase-3 in renal tissues

The enzyme activity of caspase-8, caspase-9 and caspase-3in renal tissue was assessed by colorimetric method using kits from GenScript (Piscataway, NJ, USA) following manufacturer's protocol.

2.6. Histopathological study

Renal specimens were processed for paraffin sectioning by fixing in 10% neutral buffered formalin followed by washing with water,

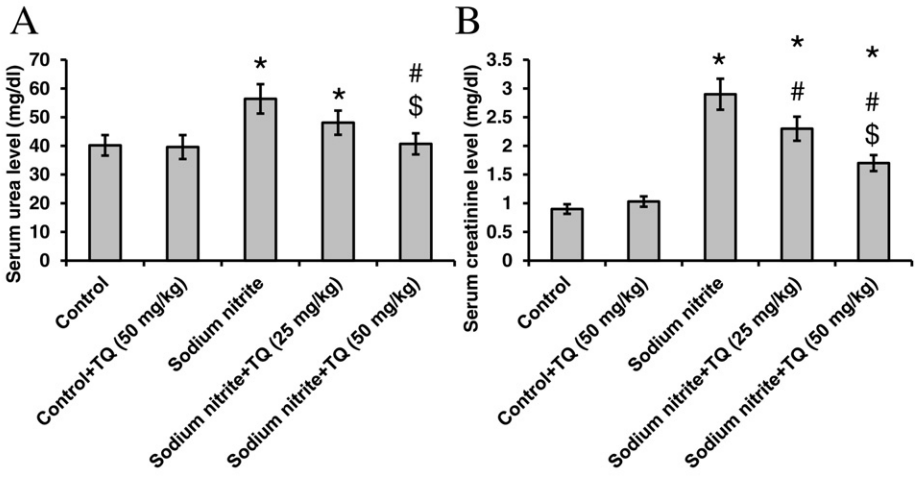

Fig. 1. Effect of oral sodium nitrite treatment (80 mg/kg) in presence or absence of oral TQ (25 and 50 mg/kg) on renal function tests. Serum A) Blood urea nitrogen (BUN). B) Creatinine. * significant against control groups at $p < 0.05$. # significant against sodium nitrite group at $p < 0.05$. $ significant against sodium nitrite + TQ (25 mg/kg) at $p < 0.05$.